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HAMILTON B. G. ROBINSON

JUNE, 1949

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EDITOR'S VIEWPOINT

Lord Chesterson has been credited with stating that much research reminded him of a blind man looking in a dark room for a black hat that wasn't there. At times we have been inclined to agree with this concept although we fully recognize the "usefulness of useless knowledge." Everett's (p. 204) contribution does not fall in with investigations of subjects which require long introductions to establish their value. He has investigated a subject of clinical argument, the relative depth of anesthesia and toxicity of two and four per cent procaine solutions. He has challenged one of our accepted ideas, i.e., that the increase in toxicity of procaine is in geometric ratio to its concentration, and he presents data to substantiate his claim. This paper should be of especial interest to the drug-approving agencies of the professions and to all using local anesthetics.

Taylor and Teamer (p. 219) discuss the properties of gold solders and suggest test requirements for such solders. This work was originally presented to the Materials Group of the I.A.D.R. and represents a field of dental research that has seldom been represented in the pages of this journal. The workers in the physical science phases of dental research desire and should have an outlet for their publications. Publication of such material is within the broad policy of the *Journal of Dental Research.*

A study of amalgams by Taylor, Sweeney, Mahler, and Dinger (p. 228) indicates that application of masticatory stress of ordinary degree within less than one hour after placement is hazardous and that an elapse of six or more hours between packing and application of stress is necessary to provide at least 50 per cent of the maximum strength. The authors used smaller specimens, more comparable in size to amalgam restorations, than most previous investigators. Mechanical triturators gave wide variation in rates of amalgamation and the investigators point out the need for more exact directions for specific alloys if consistent, good results are to be obtained with these amalgamators.

Artagaveytia-Allende (p. 242) differentiates *Paracoccidioides brasiliensis* from *Paracoccidioides cerebriformis* and points out that these blastomycosis-producing organisms may enter through an exposed pulp. While blastomycosis may appear as a remote disease to most dentists, the possibility of the causative organisms entering through oral lesions and the fact that they have been described in a periapical lesion cannot be dismissed.

Henschel and Lieber (p. 248) give their first comprehensive report on the results with ammonia-urea dentifrice (22.5 per cent carbamide; 5 per cent dibasic ammonium phosphate). For 100 individuals used, as a test group, they estimate the D.M.F./year at 2.39 on the basis of previous caries (7.8 years), and then found a D.M.F. of 1.55/year during an average 34.3 months on ammonium dentifrice. The estimate rate of 2.39 is of course extremely questionable as a rate for comparison during the future test period. The average difference of .84 cavities per year is therefore not too well established. As a "control" 50 patients with a prior D.M.F. of 2.31/year were placed on a "cosmetic" dentifrice with 2.48/year, an increase of .17 D.M.F. per year. The authors, themselves, point out that the average caries reduction of 37.5 per cent may be attributed in part "to enthusiastic oral hygiene heightened by the knowledge of possible therapeutic benefit of the dentifrice." We might add that the reduction may be attributed in part to possible additional knowledge of the effects of refined carbohydrates on dental caries rates.

Webman, Hill, and Kniesner (p. 258) found that while 70 per cent of a group of rats fed a cariogenic diet developed rat caries, only 50 per cent of a similar group with small amounts of penicillin added to their water supplies showed carious lesions. Further, when sucrose was added to the water of rats, 100 per cent developed caries but when 5 units of penicillin was also placed in each c.c. of their water, only 68 per cent had carious teeth. In the animals with penicillin and sucrose in the water *B. lactis aerogenes,* an ammonia nitrogen producer, replaced *L. acidophilus,* an acid producer, on the oral flora.

Hill and Kniesner (p. 263) used 163 institutionalized boys for a study of the effects of penicillin-containing dentifrice on oral lactobacillus counts and on the activity of dental caries. The boys were not supervised in their use of the control dentifrice (63 boys) or the therapeutic dentifrice (80 boys). Eighteen per cent of the oral lactobacillus counts continued negative. While the lactobacillus counts showed some reduction there was not significant reduction in the number of new carious lesions among those using the dentifrice containing small amounts of penicillin. The results suggest that, although penicillin appears to reduce the oral flora and in rats is an aid in caries control, under ordinary conditions of use in a dentifrice containing small amounts it is of negligible value.

Continuing the work on penicillin, White, Kniesner, and Hill (p. 267) found that the number of lactobacilli in the mouth can be reduced by conscientious use of a dentifrice containing penicillin. The small amounts of penicillin had little effect on the balance of the oral flora but reduced the numbers of bacteria. With larger doses of penicillin the Aerobacter and Escherichia groups were increased but did not persist in the flora.

White and Hill (p. 272) by an in vitro study using a modification of the agar cup method found that viable *A. aerogenes* will not inhibit *L. acidophilus* but that *L. acidophilus* will inhibit *A. aerogenes*. They confirmed the findings of Kesel, O'Donnell, Kirch, and Wach that filtrates of some strains of *A. aerogenes* will inhibit *L. acidophilus'* growth. The pH of the media greatly influenced the antagonistic reactions. The antagonistic relationships of oral bacteria is highly important in the oral cavity and may influence caries activity. The pH varies greatly in the saliva and within plaques and may change rapidly with introduction of a carbohydrate substrate. The interbacterial warfare in the plaque may determine whether caries is initiated, calculus is formed, or the tooth surface remains normal.

Pearlman, Sprague, and Best (p. 276) have observed the salivary lactobacillus counts and caries activity of mental patients before and after hypoglycemia therapy. They received the equivalent of 3 to 6.5 pounds of sugar per week in addition to their dietary carbohydrate. Those patients with low initial counts showed no significant rise in salivary lactobacilli during treatment while those with high initial counts rose progressively for five weeks, although this was not statistically significant. The authors believed the D.M.F. increase was higher than in normals although the data were not analyzed statistically. The authors suggest that intrinsic resistance was an important controlling factor, especially in those with initial low counts. A definite correlation was found between initial lactobacillus counts and sensitivity to induced insulin coma. The possibility of using the salivary lactobacillus count to predict necessary insulin dosage is discussed.

Granados, Glavind, and Dam (p. 282) fed lactic acid in food and in water to groups of hamsters on cariogenic diets and found no significant increase in carious lesions over animals fed cariogenic diets alone. Some acid decalcification of the surfaces and occlusal fossae was observed. The experimental result compares favorably with the clinical observation of decalcifications of surfaces in patients with recurring regurgitation of acid contents of the stomach or in individuals using much acid in food or drug form, but showing little or no true caries. The initial lesion of caries appears to occur in a protected area beneath a plaque as opposed to the ordinary acid decalcification on unprotected surfaces.

Dreizen, Greene, and Spies (p. 288) studied the action of a group of nitrofuran compounds which had been previously demonstrated to be bactericidal and bacteriostatic against a number of pathogens, including *L. acidophilus*, in vitro. Five of these compounds were quite effective over a fairly extended period of time. As new antibacterial drugs are developed, laboratory and clinical studies should be made to determine their effects against dental disease. Certainly, this type of study will lead to replacement of the empiricism which is still in effect in many instances.

Russell (p. 298) has compiled data from Mitchell, South Dakota, a community in which the water supply was "accidentally" fluorinated for a period of 18 months. An examination of the teeth of children now 11 to 15 years of age who had been exposed to this fluoride water before eruption of their first permanent molars showed no protection

against caries, although the fluorinization was severe enough to produce mottled enamel in 26 of the 339 children. Russell believes that his data tend to weaken the theory that fluorine deposited in enamel during calcification provides the mechanism for caries inhibition. The data are well presented and provide much food for thought.

Straub and Adler (p. 310) have investigated the saturation of coarsely powdered enamel with sodium fluoride solutions. Under conditions of their experiment they found that maximum saturation was attained in 60 minutes and that 40 per cent saturation was attained in 20 minutes. Since clinical studies have indicated that four topical applications of 4 to 5 minutes each (16 to 20 minutes, total) result in optimal reduction in caries activity it may be that saturation is not necessary. The important reaction between sodium fluoride solution and enamel, in vitro, may take place within an extremely short time, or a greater degree of saturation attained in 16 to 20 minutes may be of value. All investigators and clinicians studying and using sodium fluoride as an anticaries agent should realize that the modus operandi is still conjectural.

Slanetz and Brown (p. 313) evaluated methods for estimating the numbers of bacteria in the mouth. They found tryptose glucose blood agar useful for quantitative estimations of mouth bacteria and used tomato juice agar (pH 5) for lactobacilli. The widely used paraffin chewing method for collection of salivary samples was found advisable. Three substances (glycerite of hydrogen peroxide, Cepacol, and penicillin) were found useful in temporarily reducing the oral bacterial count, although some reduction also was noted after meals. While three 5000-unit penicillin lozenges per day were effective in reducing the bacterial count, two lozenges were not as effective, and in the one subject tested, a single daily lozenge was ineffective. This dosage factor may be an important clue in the results of Hill and Kniesner (p. 263) who had showed beneficial results in a small group under controlled use of penicillin containing dentifrice and little or no benefit in a group using penicillin presumably sporatically.

Morris and Read (p. 324) have experimented on reduction of mouth odors. They believe objectionable mouth odors to be the result of putrefaction of saliva, or in some instances, from odors of systemic origin. The use of water as a mouth rinse does not affect the putrefaction of saliva, although an antiseptic mouth wash did. Dental prophylaxis, tongue prophylaxis, use of dentifrices, and antiseptic mouth wash reduced odors of local origin while mouth rinsing with water did not. Tobacco smokers appeared to have low mouth odors other than the tobacco odor. The problem of mouth and breath odor is often given little consideration. The efficacy of different types of antiseptic mouth washes may be worthy of further study.

H. B. G. R.

A COMPARISON OF DEPTH OF ANESTHESIA AND TOXICITY OF TWO AND FOUR PER CENT PROCAINE HYDROCHLORIDE SOLUTION

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INTRODUCTION

PROCAINE with added vasoconstrictor agent is capable of providing satisfactory anesthesia for operative and surgical procedures in dentistry and possesses less toxicity than most other compounds. The question of the optimal concentration of the drug to be used in dentistry is still being argued. Following Mayer's[1] recommendation of 1924 that 1 per cent procaine was the highest feasible concentration, the Council on Pharmacy and Chemistry of the American Medical Association has recommended in its *New and Nonofficial Remedies* the use of procaine solutions not stronger than ½ per cent for infiltration anesthesia. The Council on Dental Therapeutics[2, 3] has held for many years that no solutions of procaine stronger than 2 per cent ought to be used in dentistry routinely; this stand somewhat contradicts the recommendation of the N.N.R.

There are many occasions where the 2 per cent solution proves ineffective in dentistry[4, 5] and it would seem logical to obtain better anesthesia by increasing the concentration. However fears are expressed that the use of a stronger solution, for instance of 4 per cent, would be more likely to provoke reactions or cause irritation or damage to the tissues. On the other hand, evidence has accumulated in the past tending to show that 4 per cent solutions may be used safely and with distinct advantages in certain instances.[6, 7, 8, 9] The author believes, on the basis of clinical experience, that there are definite indications for the use of the stronger procaine concentration. It would appear desirable to use procaine solutions of two different strengths in routine dental practice, probably 2 per cent and 4 per cent. The 4 per cent solution of procaine has been selected as the strongest suitable for dental anesthesia since it is the highest concentration which, allowing for added vasoconstrictor, does not become hypertonic.

Review of the recent literature on procaine suggests the need for a careful and objective reappraisal of this problem, particularly in view of newer knowledge of the intravenous administration and toxicity of procaine.[10, 11, 12] The necessary steps in such an evaluation would be: (a) an objective estimation of the completeness or depth of pulpal anesthesia following the administration of

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the 2 and 4 per cent solutions in search of measurable advantages of the higher concentration, and (b) an investigation of the influence of varying concentrations of procaine on its toxicity.

EFFECTIVENESS OR DEPTH OF ANESTHESIA OF 2 AND 4 PER CENT PROCAINE HYDROCHLORIDE SOLUTIONS AS USED FOR PULPAL ANESTHESIA BY SUBMUCOUS MAXILLARY INFILTRATION

The experimental approach in such an evaluation would involve the measurement of two elements. The first is the alteration in the rapidity of onset and the duration of anesthesia produced by varying strengths of procaine. This has been rather thoroughly studied,[13-16] as has been the influence of added vasoconstrictors on these factors.[17-22] The other element is depth or completeness of anesthesia. Since neither time of onset nor duration of action necessarily parallel the depth of local anesthesia, it was decided to attempt to measure this latter variable.

Experimental

Several new experimental procedures for comparing the effectiveness of analgesic agents have been introduced recently. One of these is the determination of the changes in the strength of electric shocks required to elicit a response when applied to an amalgam-filled tooth of a dog.[23] Since it was desired to compare the effectiveness of various concentrations of local anesthetic agents given by submucous infiltration to produce dental pulp anesthesia, the following procedure for testing the depth of anesthesia was devised.

Healthy adult dogs of either sex were used. The animal was lightly anesthetized with an intravenous injection of 20.6 mg. per kilogram bodyweight of sodium pentobarbital, a dose fortified by small repeated injections as necessary. The dog was then strapped on its back on a board and its mouth kept open by a prop. Shallow occlusal cavities were then drilled in two homologous teeth of the upper arch, using either canines or molars. The cavities were filled with a conductive paste and then tested using a weak tetanizing current. The circuit was arranged with a large indifferent electrode applied to the shaved chest and the stimulating electrode to the cavity. The strength of the stimulus was varied so as to elicit a minimal objective response (threshold) by changing the distance of the sliding secondary coil from the fixed primary coil of the Harvard Inductorium. This distance is measured by a scale graduated from 0 to 11 cm. provided on one of the sliding bars of the inductorium, where the greater the distance the secondary coil is set from the primary, the less the intensity of the stimulus.

The response observed with the threshold stimulus was a slight twitching of the skin of the lower jaw as far down as the clavicle. With increase in current, this response became more marked, with more muscles contracting. The depth of the two cavities was varied until similar minimal responses were elicited on stimulation of either side. The strength of current required, read from the inductorium, was recorded as the control threshold value.

Submucous infiltration was next performed on each side, using 2 per cent procaine with neosynephrine 1:2,500 on one side, and 4 per cent procaine with the same concentration of vasoconstrictor on the opposite side. Two c.c. of each strength solution per 100 pounds bodyweight were used, an amount comparable with that used clinically. Each cavity was then retested, and the strength of stimulus increased, if necessary, until a response was noted on one or both sides. Tests were made at intervals of five minutes following injection and continued for one hour. The minimal strength of current required to elicit a threshold response in one or the other tooth was recorded according to the reading on the inductorium and, further, the intensity of the response noted on stimulating either tooth was arbitrarily graded as 1, 2, or 3 plus. Eight such experiments were performed.

Results

Table I records the results of a typical experiment. In this dog the cavities in both teeth were prepared to such a depth as to give an equal minimal (1 plus) response when a very weak tetanizing current was used, found by setting the inductorium coil at 8.5 centimeters. Following submucous periapical injection of both procaine solutions, a 1 plus response was obtained for both teeth at 6.5 cms. for the first five-minute period. At 10 minutes after injection, a minimal 1 plus response was noted for the 4 per cent solution and a 2 plus response for the 2 per cent strength with a further increase in current intensity to a reading of 4.5 centimeters. The results obtained for each five-minute test period show that in every trial, except the first after injection, significantly less response was noted from the tooth blocked with 4 per cent procaine.

Table II summarizes the eight experiments. The amounts listed in the column under each concentration of procaine represent the total sum of the arbitrarily graded responses for all of the dog experiments for each five-minute period. For example, when each of the eight dogs were tested on the side receiving 4 per cent procaine at 10 minutes after injection, 5 dogs showed a response of 1 plus, another 2 plus, and two dogs showed no response, thus making an aggregate of plus 7. This sum was compared with the sum of plus marks for the other solutions at the identical time after injection.

An attempt has been made in the last column of Table II to present quantitatively a one-figure ratio of the depth of anesthesia produced by the two procaine solutions. This ratio has been obtained by using the formula $D = \frac{B}{A}$ where B = sum of graded response for 8 dogs with 4 per cent procaine and A = sum of graded response of 8 dogs with 2 per cent procaine and D = the relative superiority of depth of anesthesia obtained with the stronger solution. Judged by this evaluation, the ratio was always higher than 1.33 in favor of the 4 per cent solution, and at 20 and 25 minutes after injection it was greater than three.

While we do not have positive knowledge that the dogs actually suffered pain through stimulation from the active electrode, their responses, when positive, permitted the conclusion that conduction through the "blocked" nerve was occurring, and would have produced pain in a conscious animal. We were

TABLE I

COMPARISON OF EFFECTIVENESS OF 2 AND 4 PER CENT PROCAINE SOLUTIONS WHEN INJECTED SUBMUCOUSLY FOR PULPAL ANESTHESIA OF CONTRALATERAL MAXILLARY MOLAR TEETH OF THE DOG

DOG EXPERIMENT No. 6, Female Black Mongrel. Wt. 23 pounds. Date: 8/28/47. Given 3½ c.c. of Sodium Pentobarbital at 1:42 P.M.

TIME P.M.	PERIOD AFTER INJECTION (MINS.)	ESTIMATED RESPONSE FOLLOWING PERIAPICAL INJECTION OF PROCAINE HCL			INDUCTORIUM READING (CMS.)
		U.R.* 2%	TOOTH #6 PROCAINE	U.L.† 4%	
1:54	-	Control		Control	8.5
1:59	(Injection)	-		-	-
2:04	5	1+		1+	6.5
2:09	10	2+		1+	4.5
2:14	15	3+		1+	4.
2:19	20	3+		1+	4.
2:24	25	3+		1+	4.
2:29	30	2+		1+	5.5
2:34	35	2+		1+	6.5
2:39	40	2+		1+	7.
2:44	45	2+		1+	7.5
2:49	50	2+		1+	7.5
2:54	55	2+		1+	8.
2:59	60	2+		1+	8.

Identical volumes of the two procaine solutions, 0.46 c.c. each, were injected. Both solutions contained neosynephrine 1:2,500. For explanation of testing procedure see text.

*Upper right molar #6.

†Upper left molar #6.

TABLE II

SUMMARY OF RESULTS COMPARING DEPTH OF PULPAL ANESTHESIA OBTAINED WITH 2 AND 4 PER CENT PROCAINE HYDROCHLORIDE* IN EIGHT DOGS

PERIOD TESTED AFTER INJECTION (MINS.)	SUM OF ARBITRARY RESPONSE UNITS AFTER INJECTION OF 4% PROCAINE HCL	SUM OF ARBITRARY RESPONSE UNITS AFTER INJECTION OF 2% PROCAINE HCL	RATIO OF DEPTH OF ANESTHESIA
5	6	8	1.33
10	7	14	2.00
15	7	13	1.85
20	5	16	3.20
25	5	16	3.20
30	7	19	2.71
35	6	16	2.66
40	6	18	3.00
45	6	15	2.50
50	6	17	2.83
55	8	16	2.00
60	8	17	2.12

*Both solutions contained Neo-synephrine 1:2,500.

aware also that there exists a question as to whether conclusions based solely upon the reactions of animals to a particular stimulus constitute a sufficient index for the degree of anesthesia.[24] To determine whether our findings could be corroborated in human patients, the following clinical tests were performed.

Clinical Tests in Human Patients

Volunteers were selected who had in their upper arch homologous vital teeth that were to be extracted in the course of prosthetic restoration. Canines or teeth posterior to canines were used to avoid overlap of the field of anesthesia.

The teeth were radiographed and then prepared for a test in a manner similar to that described in the animal experiments with some minor points of difference in the arrangement:

1. No barbiturates were used preliminarily.
2. The indifferent electrode was connected with the palm of the patient's hand.
3. Volume of solution used was 1 c.c. for each 100 pounds of patient's bodyweight.
4. We tested preliminarily and after injection every 5 minutes for bare perception of stimulation and then, immediately afterwards, for slight but definite pain.

Nine such experiments have been run. Solutions used were the same as in the animal experiments, that is, procaine hydrochloride 2 per cent and 4 per cent, both with neosynephrine 1:2,500. The solutions for these tests as well as for the animal experiments were freshly prepared; their pH was approximately 4.8. The higher and the lower concentration have been used on either side in these cases. When in these tests we raised the intensity of the stimulus above that required for eliciting a slight but definite pain, progressively more marked flinching and twitching of the muscles of the face could be observed.

The results show significantly deeper anesthesia on the side anesthetized with the stronger solution. It is interesting that in these tests the period of most profound pulpal anesthesia seems to have passed about 30 minutes after injection, as signified in Table III by the decrease in current intensity from 0 to 4 necessary to elicit pain even in the side anesthetized with the stronger solution. Slight pulpal anesthesia was present on this side for the balance of the testing period. On the side anesthetized with the weaker solution pulpal anesthesia had disappeared about 40 minutes after injection.

These clinical tests have also shown the correctness of our interpretation of the animal experiments, namely, that the flinching and twitching seen were responses to stimulation of an intensity experienced as pain. Table III records the results of a typical test.

Discussion

Previously, experimental data comparing the potency of anesthetic agents have been derived from the routine testing of the rabbit cornea, the frog or guinea pig sciatic nerve, the human dermal wheal, and by other procedures.[15, 19, 25-29] Transfer of data obtained from these experiments directly to clinical dentistry or medicine involves two assumptions: (a) that onset and duration of anesthesia indicate depth, and (b) that different tissues respond similarly to subcutaneous or submucous injection with procaine solutions of identical concentrations. The first of these assumptions arose because of the obvious difficulty of quantitatively measuring pain. Recently, however, Wolff, Hardy, and Goodell,[30] and others have devised methods of comparing analgesics without making this assumption, and the need for similar comparisons in the field of local anesthesia is apparent. It is a common clinical observation that local anesthesia may be inadequate for the contemplated procedure, yet last for

TABLE III

COMPARISON OF CLINICAL EFFECTIVENESS OF 2 AND 4 PER CENT PROCAINE SOLUTIONS† IN SUBMUCOUS INFILTRATION FOR PULPAL ANESTHESIA OF CONTRALATERAL HUMAN MAXILLARY FIRST BICUSPIDS

3/13/48 EXP. NO. 3	RIGHT SIDE 2%		LEFT SIDE 4%	
TIME	INDUCTORIUM READING (CMS.)	PATIENT'S SENSATION OF STIMULUS	INDUCTORIUM READING (CMS.)	
8:40	10.5	perceptible	10.5	control
	10.0	painful	10.0	control
8:43	injections			
8:48	6.5	perceptible	2.0	
	5.0	painful	- *	
8:53	6.0	perceptible	1.0	
	5.0	painful	- *	
8:58	6.0	perceptible	1.0	
	5.0	painful	- *	
9:03	6.0	perceptible	- *	
	5.2	painful	- *	
9:08	7.0	perceptible	1.0	
	6.0	painful	- *	
9:13	8.0	perceptible	5.0	
	7.5	painful	4.0	
9:18	9.3	perceptible	7.0	
	8.8	painful	6.2	
9:23	10.3	perceptible	8.3	
	10.1	painful	7.9	
9:28	10.4	perceptible	8.5	
	10.2	painful	8.1	
9:33	11.0	perceptible	9.2	
	10.5	painful	8.8	
9:38	11.0	perceptible	9.5	
	10.5	painful	9.0	
9:43	10.8	perceptible	10.0	
	10.4	painful	9.5	
9:48	11.0	perceptible	10.2	
	10.5	painful	9.8	

Patient's weight, 140 pounds. Amount of solution used on either side was 1 c.c. per 100 pounds, that is, 1.4 c.c. After changing the depth of preparation in the two teeth so as to obtain an equal response on either side, the reading on the inductorium was 10.5 for bare perception and 10 for slight pain. The first upper bicuspids were tested.

*No pain response could be elicited.

†Both solutions contained Neo-synephrine 1:2,500.

long periods. Moreover, with the use of vasoconstrictors, duration of local anesthesia becomes less dependent upon the factor of concentration of the anesthetic agent.

The second assumption is similarly not tenable. There is no reason to assume that simply because a ½ per cent solution of procaine will suffice for subcutaneous infiltration anesthesia and block pain receptors, that the same concentration will also effectively block the sensory fibers of a nerve trunk some distance from the site of injection. In the former case it is only necessary for the agent to act upon scattered single receptors, while with submucous administration for pulpal anesthesia, the solution must first penetrate the outer compact layer of the alveolar process; during this comparatively slow passage considerable dilution probably occurs. This weakened solution then has to act not on scattered pain receptors as in the mucosa but on the sensory fibers and their sheaths of the moderately large pulpal nerve trunk, and may prove ineffective unless a solution of higher concentration has been administered. In other words,

in submucous infiltration for cavity preparation, we are actually attempting block anesthesia of the pulpal nerve trunk but are injecting the solution in an area separated from that trunk by compact bone; it is probably this physical obstacle that necessitates the increase of concentration in conjunction with the further fact that a nerve block is attempted requiring stronger solution than that essential for terminal anesthesia. Furthermore, the nervous structures of the dental pulp probably have a pain and anesthetic threshold quite different from that of the periodontal membrane, that of bone, mucous membrane, or the skin. It would seem therefore, that the determination of comparative anesthetic potencies by methods employed in the past have but little significance when applied to dental pulp anesthesia by submucous infiltration.

Goetzl, Burrill, and Ivy[23] have used the dental pulp as a test organ to measure effectiveness of analgesic and hypnotic drugs such as morphine and acetylsalicylic acid. They state that "the tooth pulp possesses specific pain receptors and there is good evidence that receptors of other afferent systems are absent." Histological studies reported by Noyes, Schour, and Noyes[31] and Orban[32] support this concept. With the technic devised in this study, it is felt that testing of the dental pulp provides a quantitative procedure for comparing the effectiveness of various concentrations of different anesthetic agents also. Although the data summarized in Table II permit only a relative estimation of the anesthetic depth obtained with the two procaine solutions, they suggest that superior depth of pulpal anesthesia is obtained with the 4 per cent solution of procaine hydrochloride.

COMPARISON OF TOXICITY OF 2 AND 4 PER CENT PROCAINE HYDROCHLORIDE SOLUTIONS

Introduction

In 1924 Mayer[1] undertook a review of the question of toxicity of various local anesthetic agents. His recommendation, not to exceed a strength of 1 per cent of procaine hydrochloride for purposes of infiltration, was based on a thorough study of fatalities that had been caused by a number of local anesthetic agents. Out of the 43 fatalities reported, only 2 could be ascribed by him to procaine. One of these two cases, a tonsillectomy patient, received infiltration anesthesia with 33 c.c. of ½ per cent procaine solution with epinephrine 1:10,000. He died on the operating table. On autopsy status lymphaticus was demonstrated. The second case mentioned received ½ per cent procaine solution subcutaneously in a case of extensive burns; no amounts of procaine were mentioned nor presence or absence of vasoconstrictors. The patient died within 30 seconds after injection. Hatcher and Eggleston[33] mention 2 procaine fatalities. Both their cases were spinal anesthesias, in neither case was concentration mentioned, and in one of the 2 cases no total dose of procaine was given. The fatality reported by Hansen[34] was a patient with severe asthma whose stellate ganglion was blocked with from 20 to 80 c.c. of 1 per cent procaine hydrochloride 5 times within 20 days. She died of respiratory failure at the sixth injection (epidural injection?).

As pointed out by Hertzler,[35] none of these cases is conclusively due to the toxicity of procaine alone and they certainly do not afford an adequate basis for comparing the toxicity of procaine in any specific concentrations. Drug sensitivity and shock probably account for the majority of fatalities mentioned above.

It might be well in this connection to consider briefly the following figures. There are about 120,000 physicians in this country and 70,000 dentists. Let us surmise that each physician gives 1 procaine injection daily, that each dentist gives 5 procaine injections daily and that a work year has 300 days. A total number of 141,000,000 procaine injections are then given in this country each year. Even allowing for the use of substitutes, well over one hundred million procaine injections are probably given in the U.S. alone annually. That includes concentrations of ½ per cent up to spinal injections of 10 per cent. In the light of this information the list of procaine fatalities does not appear impressive.

The *New and Nonofficial Remedies* advise that "the total amount injected should not exceed 200 mg. of the drug." For dental purposes usually 2 c.c. of anesthetic solution are used; occasionally 3 or 4 c.c. may be injected. The drug amounts administered, therefore, are well below the maximum dosage recommended, even when a 4 per cent solution is used.

When any material is injected subcutaneously or submucously, certain histological changes may occur. Whether the stronger solution will cause tissue damage has been answered by Braun[15] and Farr.[36] They state that even a 10 per cent solution of procaine hydrochloride is absorbed after injection without leaving any trace of tissue irritation.

It might be well to point out here that procaine was synthesized by Einhorn[37] in 1905 and that Braun[18] had recommended the addition of epinephrine to local anesthetic agents in 1903. Procaine, therefore, from the date of its discovery on has almost always been used in combination with vasoconstrictor. It is most probable that the majority of the toxic symptoms seen after the administration of local anesthetics are due to the vasoconstrictor rather than the anesthetic agent.

Dramatic changes in blood pressure occur when epinephrine is injected intravenously even in minute amounts; they account undoubtedly for the occasional case of perspiration, clammy skin, or fainting seen in the dental chair.

Dentists, in their overwhelming majority, use cartridge syringes which, while convenient otherwise, generally do not permit aspiration to test for accidental intravenous injection. Recently Nevin[38] has described a cartridge syringe permitting aspiration; however, in the hands of the writer the syringe permits aspiration only by using undue pull, thereby making it difficult to maintain the position of the needle point.

That we probably have been wrongly indicting the toxicity of the comparatively innocent component, namely, that of procaine instead of that of the vasoconstrictor, has been demonstrated in recent years in the clinical use of procaine hydrochloride, of course without vasoconstrictor, for intravenous analgesia and anesthesia. Allen et al.,[10, 11] and Bigelow[12] have reported the use

of as much as 425 c.c. of a 1 per cent solution intravenously for general anesthesia over a period of one and three-fourths hours. The first 8 c.c. of the solution may be administered over a period of just one minute. At the University of Oregon Medical School up to 10 grams in 1 per cent solution given intravenously have been used within one hour.[39] If one considers now that animal experiments have established that intravenous administration is approximately 10 times as toxic as subcutaneous administration, one cannot help wonder about the arbitrary maximal dose of 200 mg. for subcutaneous use prescribed in the N.N.R.

New research in recent years is also forcing changes in our concept of the fate and ultimate disposal of procaine in the body, a question very important for the understanding of the toxicity of this substance.[40, 41]

As for data in the literature pertaining to the toxicity of procaine when used for local anesthesia in different concentrations, we find repeated references to the "geometric ratio of increase in toxicity of procaine hydrochloride solutions."[42, 43] By this formula the authors refer to a progression according to which 1 c.c. of a 2 per cent solution of procaine hydrochloride is not twice but four times as toxic as 1 c.c. of a one per cent solution. An inquiry on this subject at the Council of Pharmacy and Chemistry of the American Medical Association brought a telegram from Walton Van Winkle, Jr.,[44] with a list of references. Study of these references follows: Eggleston and Hatcher,[45] in their toxicity studies, used concentrations of between 5 and 20 per cent of procaine hydrochloride, but no attempt was made to elaborate any difference in toxicity between any of these concentrations. Meeker and Fraser[46] used one concentration only, namely, 0.5 per cent. Schmitz and Loevenhart[25] give drug amounts only, but no concentrations at all. Dunlop[47] deals with the fate of procaine rather than its toxicity. Schamp[13] used 2 per cent solutions only. Issekutz and Kovari[48] dealt not with toxicity but with metabolism of procaine. Epstein and Silver's[49] article, contains a table comparing the toxicity on subcutaneous administration of several commercial 2 and 4 per cent procaine solutions containing different vasoconstrictors and preservatives; these additions diminish the usefulness of their findings for our study of the toxicity of procaine hydrochloride alone in different concentrations. It is interesting enough though, that in this table the 4 per cent solutions appear less than doubly toxic on the average, when compared with the 2 per cent solutions containing a comparable amount of vasoconstrictor, and when the comparison is based on solutions representing identical amounts of procaine hydrochloride. For their determination of toxicity of procaine hydrochloride without vasoconstrictor, these authors used 2 per cent solution only.

No experimental or clinical proof for the theory of the geometric progression in toxicity of procaine had been found. Waters[50] suggested revision and re-examination of this problem. We have felt therefore the necessity of a comparison of the toxicity of 2 per cent and 4 per cent procaine hydrochloride in order to establish the influence, if any, of concentration of this drug on its toxicity.

Experimental

Rapid intravenous injection into the ear vein of male white rabbits was used for the establishment of the intravenous LD_{50} for 2 and 4 per cent isotonic procaine hydrochloride solutions. Animals of 1.3 kilograms to 2.8 kilograms weight were used, the majority of them around 2 kilograms body weight. The drug was administered within periods of from 18 to 25 seconds to simulate clinical conditions where the drug may be administered at that rate intravenously inadvertently. This mode of administration is considered the best yardstick for establishment of the toxicity of procaine hydrochloride.[46, 51] Table IV shows the results.

TABLE IV

MORTALITY RATE IN RABBITS GIVEN RAPID INTRAVENOUS INJECTIONS OF 2 AND 4 PER CENT SOLUTIONS OF PROCAINE HYDROCHLORIDE*

DOSE	2% PROCAINE HCL	4% PROCAINE HCL
MG./KG.	NO. DYING/NO. USED	NO. DYING/NO. USED
40	0/4	0/4
45	1/4 (25% died)	0/4
50	5/10 (50% died)	5/9 (55% died)
55	7/8 (87% died)	4/6 (66% died)
60	4/4 (100% died)	4/4 (100% died)

*Containing no vasoconstrictor.

Rapid subcutaneous injection in Swiss male white mice has been used for the establishment of the LD_{50} in the 2 concentrations for this usual mode of administration.[26] Animals of 13 grams to 32 grams weight were used, the majority of them around 20 to 25 grams weight. Results are shown in Table V.

TABLE V

MORTALITY RATE IN SWISS MALE WHITE MICE GIVEN SUBCUTANEOUS INJECTIONS OF 2 AND 4 PER CENT SOLUTIONS OF PROCAINE HYDROCHLORIDE*

DOSE	2% PROCAINE HCL	4% PROCAINE HCL
MG./KG.	NO. DYING/NO. USED	NO. DYING/NO. USED
800	2/20 (10% died)	3/20 (15% died)
900	14/28 (50% died)	15/28 (54% died)
1000	14/20 (70% died)	16/20 (80% died)

*Containing no vasoconstrictor.

In all toxicity studies we used animals only once, notwithstanding reports by Schamp, Schamp, and Tainter[52] who reinjected the survivors after 10 days and found no significant difference in the percentage mortality between fresh animals and those used for a second time. To minimize any effect on our results from slight variations in the different batches of animals, we injected approximately the same number of each batch with 2 per cent as with 4 per cent solution.

Results of Toxicity Studies

The LD_{50} for rapid intravenous injection (administration time 18 to 25 seconds) into the ear vein of the rabbit was found to be 50 mg./kg. for the 2 per cent solution and only very slightly less than 50 mg./kg., but definitely more than 45 mg./kg. for the 4 per cent solution.

The LD_{50} for rapid subcutaneous injection near the tail in Swiss male white mice was found to be 900 mg./kg. for 2 per cent solution, and slightly less than 900 mg./kg. but definitely more than 800 mg./kg., for 4 per cent solution.

Our findings both in the rapid intravenous and in the subcutaneous tests with procaine hydrochloride rather definitely show that there is no or only a very insignificant increase in toxicity as expressed in LD_{50} when the solution administered is increased in concentration from 2 to 4 per cent as long as the drug amount is kept constant.

Discussion of Toxicity Studies

A very great number of findings on the toxicity of procaine hydrochloride, when administered subcutaneously or intravenously in laboratory animals, have been published in the past 43 years. Insufficient or no regard has been given in these investigations to dependence of toxicity on concentration. For convenience of administration, studies in the past have used generally a very high concentration, as 10 per cent for the rapid intravenous route; neither do these studies as a rule specify exactly the period of time in seconds within which this rapid administration took place. Both the factors of concentration and that of the exact period of administration time are most important in the determination of toxicity of a drug as rapidly disposed of as procaine. That probably explains the wide divergence in toxicity figures (LD_{50}) quoted in standard monographs,[28] particularly in regard to intravenous administration. We have not been able to find in the literature any references to a comparison of toxicity in procaine solutions of various strength, 2 and 4 per cent in particular, when identical drug amounts were used.

Instead we have encountered arbitrary standards as that of the "geometric ratio of increase in toxicity of procaine," a theory taught even today in many schools. Our findings show that this theory cannot be substantiated by LD_{50}'s in laboratory animals.

Toxicity might however express itself in untoward symptoms in man only. Lovestedt[8] has administered 4 per cent procaine in a series of 500 cases of heart disease, treated for dental conditions at the Mayo Clinic, and shown the absence of any untoward symptoms with these poor risk patients.

In view of the experimental findings on toxicity presented in this paper and the clinical studies of Lundy,[6] Stafne,[7] Farquhar,[9] and especially those of Lovestedt,[8] it is felt that the use of 4 per cent solution in dentistry is safe.

Conclusions

The necessity for more profound anesthesia than that obtained by administration of 2 per cent solutions of procaine hydrochloride is frequently felt by dental clinicians. Tainter, Throndson, and Moose[4] have shown that an average of 5.5 c.c. of 2 per cent solution containing epinephrine 1:50,000 was required to obtain adequate clinical dental anesthesia. Out of the 344 cases examined in this study, 21 per cent showed inadequate depth of anesthesia even with this rather large drug amount and volume. Winter and Tainter[5] reported a clinical comparison of 2 and 3 per cent procaine solutions, both containing epinephrine

1:50,000. In this study they state that an average of 2.8 c.c. of the 3 per cent solution and an average of 3.4 c.c. of the 2 per cent solution was required to obtain adequate anesthesia. The amount of 3.4 c.c. of the 2 per cent solution needed in this study varies considerably from the amount of 5.5 c.c. of the same solution needed for identical purposes by the same author in his previous work. Winter and Tainter stated that an average of 2.8 c.c. of the 3 per cent solution causes a smaller incidence of nervous perspiration and of respiratory changes than an average of 3.4 c.c. of the weaker solution, but that it doubled the frequency of tremors and greatly increased the incidence of nervousness. These symptoms are of course very much of a subjective nature and hard to measure. The authors also found a shorter duration of anesthesia for the stronger solution, supposedly caused by the vasodilator effect of the procaine that offset the vasoconstrictor effect of the epinephrine more potently when a stronger solution of procaine was used. These findings differ from Sollman's[19] and Braun's[15, 18] reports, who found an increase in duration of anesthesia when the concentration of procaine hydrochloride was increased and that of epinephrine was kept constant. The durations given by Winter and Tainter are 136 minutes for the 3 per cent solution and 190 minutes for the 2 per cent solution. They stated however that with the 3 per cent solution there was a significantly higher incidence of complete anesthesia and only a small number of reinjections. These authors considered anesthesia as adequate when it permitted the completion of the operation. However, Tainter, Throndson, and Moose[4] did not specify the type of operative or surgical procedures performed for which these anesthesias were administered beyond saying that their observations were made on patients subjected to the ordinary operations in oral surgery, mostly extractions.[4] It can be deduced that Winter and Tainter's[5] cases were also cases of oral surgery only.

Tainter and his co-workers were probably attempting to determine the best single all-round anesthetic solution for oral surgery. His findings therefore have but small significance for the determination of depth or duration of pulpal anesthesia. It has been the contention of this writer that not one but at least two concentrations ought to be used routinely for all the various operative and surgical procedures of a dental practice, namely a 2 per cent and a 4 per cent solution of procaine hydrochloride.

In our own tests we have found not a decrease but rather an increase in the length of pulpal anesthesia with 4 per cent procaine as compared with 2 per cent solution containing identical amounts of vasoconstrictor. The explanation for this divergence possibly lies in the difference of the end points taken and tissues tested by other authors and by this writer. Winter and Tainter judged duration by recurrence of sensation as determined by the use of an explorer on the mucous membrane. In pulpal anesthesia for cavity preparation, one of the indications for the stronger solution advocated by this author, obviously the anesthesia or lack of anesthesia of the mucous membrane is immaterial.

Our own observations extended over one hour only, the length of a fairly long dental appointment. Anesthesia of good depth and a duration of one hour

is desirable. It is of questionable practical advantage to have protracted anesthesia after completion of the operation and is rarely welcomed by the patient. A duration of two hours as produced by 3 per cent solution in Winter and Tainter's study would be actually preferable to one of three hours as produced by 2 per cent solution by these authors. Thus Winter and Tainter have found in comparing 2 and 3 per cent procaine solutions that for a dental appointment of ordinary length the more concentrated solution gives a deeper, hence more satisfactory, anesthesia. This agrees fully with our findings in comparing 2 and 4 per cent solutions of procaine.

The use of repeated injections as advocated by Winter and Tainter for the purpose of obtaining sufficient anesthesia also introduces an element of chance as far as the determination of toxicity and duration are concerned. It has been shown that the toxicity of procaine increases after injection of an unchanged total drug amount in several places instead of into one depot.[53] On the other hand, toxicity is bound to decrease when repeated smaller dosages are administered until sufficient depth of anesthesia is obtained, even when only as little as five or ten minutes elapse between the original injection and the second supplementary dose, because of the rather rapid elimination of the drug from the system. As far as duration of anesthesia is concerned it cannot very well be measured accurately if repeated injections at arbitrary intervals are given.

In the first part of our study, then, we have shown that experimentally and clinically 1 c.c. of a 4 per cent solution of procaine hydrochloride containing Neo-Synephrine 1:2,500 gives a more profound anesthesia of the dental pulp than 1 c.c. of a 2 per cent solution, containing an identical concentration of Neo-Synephrine, when administered submucously in the upper jaw.

In the second part of this paper we have shown that without added vasoconstrictor a 4 per cent solution of procaine hydrochloride is not much more toxic as a 2 per cent solution in laboratory animals, when drug amounts are kept constant. Four per cent solution is just about 10 per cent more than twice as toxic as 2 per cent solution when equal drug amounts are used. A "geometric ratio of increase in toxicity of procaine hydrochloride" could not be shown to exist. Drug amounts used in either concentration in dentistry, let us say up to 4 c.c., are well within the strictest permissible limits of 200 milligrams.

We wish to emphasize however that we do not recommend universal use of 4 per cent solution in dentistry but rather the establishment of indications for the use of the stronger solution which may then be used by the profession with confidence. The 4 per cent solution should be used mainly in maxillary infiltration anesthesia for cavity preparation and also in any local anesthesia, block, or terminal, for the extraction of a tooth with involvement of the apex, the periodontal membrane, or the curetting of granulomatous apical foci.[54] The 2 per cent solution should be used in all other operations.

SUMMARY

1. An objective experimental laboratory procedure for comparing depth of anesthesia after administration of different local anesthetic agents is described.

2. Experimental and clinical tests are reported corroborating the applicability of such a method in comparing intensity of anesthesia for different concentrations of procaine hydrochloride.

3. The results of this study indicate a greater depth of anesthesia of the dental pulp following the submucous administration of 4 per cent procaine hydrochloride solution as compared with the 2 per cent solution with both solutions containing Neo-Synephrine 1:2,500.

4. Toxicity of procaine hydrochloride without added vasoconstrictor is not materially dependent upon concentration in laboratory animals when 2 and 4 per cent concentrations are used, the increase in toxicity being less than 10 per cent when the concentration is increased from 2 to 4 per cent, and drug amounts are kept constant.

5. The theory of the "geometric ratio of increase in toxicity of procaine" is untenable in laboratory animals when death is used as an end point.

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GOLD SOLDERS FOR DENTAL USE

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IF published data are taken as a basis for judgment, gold solders for dental use have apparently been given less attention than any other type of gold alloy used in dentistry. In all probability this class of materials has received far more consideration than a survey of the literature would indicate, since there have been many changes in the types of solders found on the market and new formulae are constantly appearing in the various standard lines of dental golds.

Many individuals have very definite ideas as to the proper performance standards for solders, but in many cases their opinions are based upon their own individual technical processes and upon equipment which few would consider standard. Manufacturers have found it difficult to satisfy all individuals with any one type of solder and probably never will be able to do so since irregularities in methods of use can ruin the qualities of solders, no matter how fine their performance under the proper conditions.

A list of physical characteristics generally considered desirable in solders is given below. They are enumerated in an approximate order of importance:

1. Easy flowing.
2. Free flowing.
3. Freedom from pitting.
4. Strength approximating or exceeding that of the underlying structure.
5. Color to match the materials with which they are used.
6. Building qualities which permit the development of desired shapes by partial fusion.

To these must be added the chemical property of:

7. Freedom from corrosion or staining in service.

The terms Easy flowing and Free flowing are often used synonomously, but on more careful consideration seem to be applied to two separate conditions. Easy flowing may be considered as referring to the location of the fusion range which should, of course, be sufficiently below that of the materials to be soldered that all danger of melting any portion of them during the soldering operation may be avoided and, at the same time, it must be low enough to permit easy manipulation with the equipment found in the dental office. Free flowing materials are those which flow freely once they are melted and this term covers those characteristics influenced by fluidity and

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surface tension and describes the property which enables solders to spread over clean metal surfaces, to pull themselves into fine openings, and to follow along metals at points of contact.

Freedom from pitting in solders appears to be a condition highly desired but seldom entirely obtained. Pitting may be, and often is, almost entirely mechanical and is the result of incomplete or improper fluxing. These mechanical factors are influenced by the width of the melting range of the solder, its viscosity, and the viscosity of the flux used. The composition of the solders may also be a factor, in that solders containing large percentages of low fusing base metals may have a portion of these metals converted to vapor by overheating and so be a source of a type of pits very aggravating to the user.

The strength of solders is important in that solder joints are often small in proportion to the size of the other portions of dental restorations and must carry correspondingly higher stresses. The hardness and strength of gold solders increase with decreasing fineness and fortunately, dental solders, being of lower karat than the materials upon which they are used, are normally as strong as or the stronger of the two and the user seldom needs to consider strength in selecting a solder for use. Solders above .650 in fineness should not be used in small sections carrying heavy stresses.

Color is often mentioned as an important property of solders, but in practice it appears that the importance of color in solder is largely a matter of whether or not it is inconspicuous in the polished restoration ready to be placed in service, rather than after the restoration is inserted in the mouth. Polished gold-colored solders usually match the color of dental casting alloys sufficiently well to avoid comment, despite the variations in color found in commercial solders. It might be pointed out that they would be even less noticeable in the average case if users were careful to use only the amount of solder necessary to the contruction of the case and no more.

The characteristic of a solder which permits it to be maintained in a semiplastic state and to be built into desired shapes depends, not only fundamentally on its basic composition and the width of its fusion range, but to a greater extent upon the ability of the user to control properly his soldering flame. This characteristic is not so important today as it was in the period when "solder" bridges predominated in the field of bridgework.

Aside from the characteristics desired in individual solders, consideration must also be given to the comparative melting ranges of solders considered as a series since many dental restorations are constructed by step or progressive soldering operations which require the use of solders which have fusion ranges sufficiently separated to permit these operations to be carried out conveniently and safely. The actual differences in melting ranges required are less than might appear from fusion range data but, in selecting solders from a series for use, materials varying in fineness by 50 to 70 parts per thousand should be chosen.

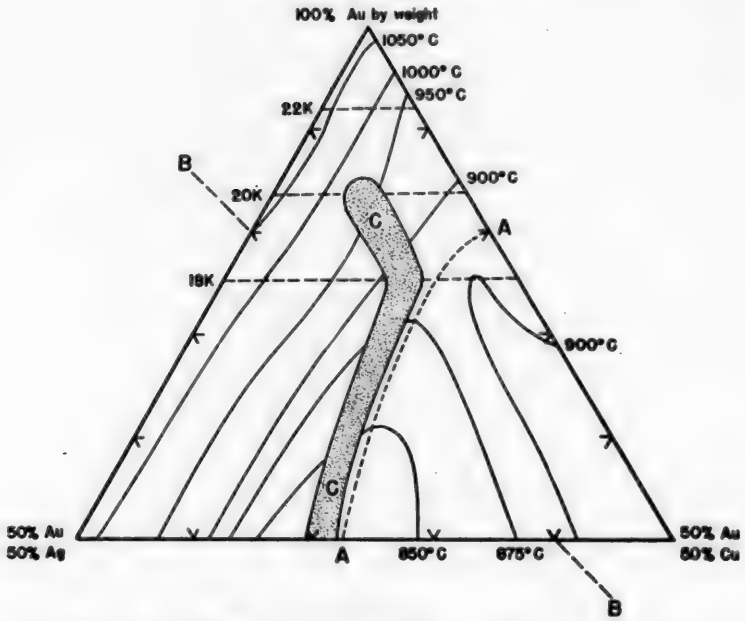
The relative resistance of solders to corrosion and staining in service is difficult to determine by testing the solders themselves, no satisfactory and indicative corrosion having been developed for dental golds. It may be assumed that the performance characteristics of the higher karat solders are better than those of the lower karat materials, and the use of the higher grades of materials is to be recommended on this basis; but as the physical characteristics must be considered carefully in selecting solder, the physical advantages accruing through the use of lower karat solders in many cases outweigh the possible greater corrosion resistance of high karat alloys.

Government agencies have been interested in acceptable specifications for dental solders, and, in connection with development work on some new solder formulas, it seemed desirable to outline a basis for a solder specification which might be effective in defining solders of acceptable working qualities as well as being based on comparatively simple test methods.

Since the development of practical test methods and specifications for solders requires a basis of fundamental information, a considerable number of solder formulas were studied to develop such a basis. These materials were essentially alloys of gold- silver- and copper to which varying proportions of zinc, tin, and phosphorus had been added, and it seemed advisable to consider the ternary system as a base in comparing these materials.

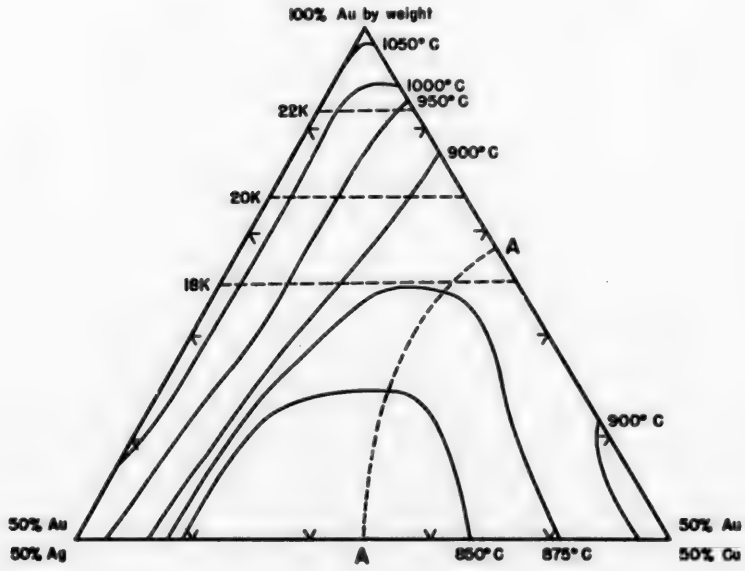
The data of L. Sterner-Rainer[1] provided a satisfactory basis for the desired comparison and they have been summarized in convenient form in two ternary diagrams (Figs. 1 and 2) which show the liquidus and solidus of the series by means of isotherms which have been constructed by interpolation from the original melting range data. In each of the diagrams the approximate location of the series of minimum fusing alloys which lie between the copper-silver eutectic and the minimum fusing gold-copper composition are shown by the dotted lines *A-A*. The compositions of the plate alloys commonly used in dentistry are also indicated by horizontal lines. In practice the silver content of plate golds usually exceeds the copper content. To avoid unnecessary complication of these diagrams the solder compositions studied, reduced to their basic gold-silver-copper proportions, are indicated in a third diagram (Fig. 3).

A study of the first two diagrams shows that the melting ranges of dental plate golds could be decreased by the addition of copper alone, but experience has shown that the lowering obtained was insufficient to provide the low fusing alloys desired for solders. The desired decrease in the fusion range has generally been accomplished by combining the effects of lower gold content, increasing the copper content, and by the addition of low fusing base metals to the original plate formulas. The resulting alloys are commonly designated as solder for specified karat plates or by their fineness in parts per thousand of gold. Many of the casting alloys now used are sold by trade names or brand designations and their karat values are not given. It is prefer-



Liquidus. Gold-Silver-Copper- Series (Sterner-Rainer)

Fig. 1.



Solidus. Gold-Silver-Copper Series (Sterner-Rainer)

Fig. 2.

able to have the fineness stamped upon each piece rather than having them marked "For 18K," or perhaps 18-18-18, these latter markings being only an indefinite indication of usage.

A study of one series of solders to determine the effects of added base metals (zinc and tin) gave roughly a 10° C. lowering of the liquidus and a 24° C. lowering of the solidus for each one per cent of base metals added when the fineness was .625 (62.5 per cent of gold) or lower. The lowering of the liquidus of higher karat solders was about 5° C. greater for similar additions while the effect on the solidus was about the same as in the lower karat materials.

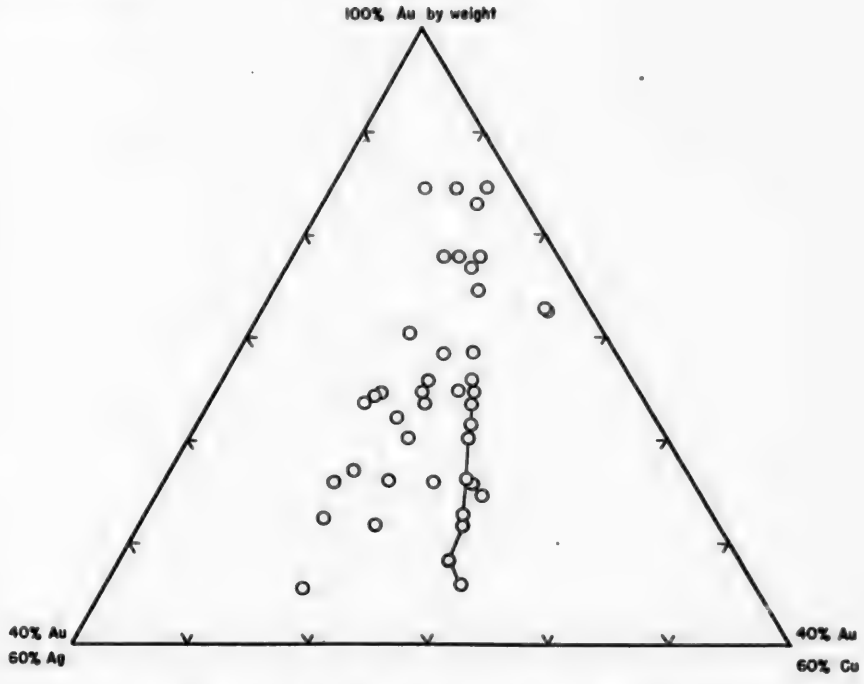


Fig. 3.

These figures are only approximations when applied to all types of solders since effects of the added zinc, tin, and phosphorus depend upon the proportions of each and these values vary widely in commercial solders. The observed values were, however, sufficiently constant to serve as a basis for reasonably accurate predictions as to the qualities and properties of solders of other basic compositions with similar fusible metal additions.

In the first diagram (Fig. 1) the line *A-A* divides the compositions into two groups which in practice serve as a basis for two types of solders. When alloys with constant amounts of the low fusing constituents are observed, those

whose basic compositions lie to the right of the dividing line seem more difficult to flow on current dental alloys despite the fact that their melting ranges may be lower than some of the alloys lying to the left of the dividing line.

Their sluggish flowing qualities may be explained if consideration is given a vertical section of the gold-silver-copper diagram taken along *B-B* in Fig. 1 and shown in Fig. 4. From this diagram it will be seen that solders of this type are higher fusing than alloys intermediate between their compositions and that of a plate alloy in which they might be used. When they are fused, the first reaction seems to be to absorb gold or perhaps both gold and silver from the plate until the lowest fusing mixture is reached at which time the solders flow freely. As a time factor is involved, operators may feel that the solder needs extra heat to make it flow. Actually overheating may occur and it will aggravate the tendency of this type of solder to penetrate the plate and will make it appear thin and watery when it finally starts to flow. Solders of this type have very satisfactory building characteristics because of their initial "sticky" tendencies. They are very useful in building solder pontics and inlay contacts. Their color is on the dark side because of the higher copper content.

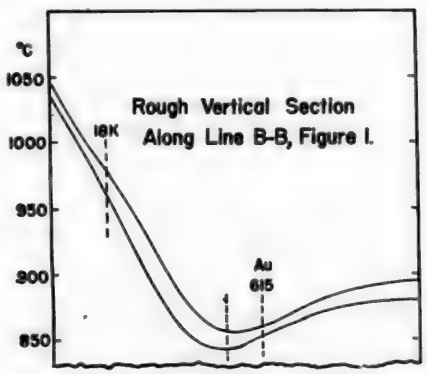


Fig. 4.

Solders whose basic compositions lie to the left of the lowest melting compositions are much easier to handle in ordinary dental soldering operations, flow starting as soon as melting becomes apparent. Assuming that it is in this group that the most satisfactory dental solders will be found, further consideration of the basic diagram would suggest that the best possibilities would lie close to the minimum fusing compositions, and that, if a series is to be considered, compositions should be so selected that a line drawn through their basic compositions would parallel the low fusing composition line (*A-A*) closely until above 72 per cent gold when it should turn to the left and roughly parallel the gold-copper base line. This line would cut isotherms roughly at right angles, and the compositions through which it passes would give the maximum increases possible in melting ranges with necessary variations in fineness. It is possible to select a series of solders whose compositions lie along an isotherm and so defeat the purpose behind the use of varying fineness in solders as an

entire series may have approximately the same melting range. In Fig. 3 a line has been drawn through the basic compositions of twelve solders which, despite a .150 variation in fineness, have a maximum difference in melting ranges of about 25° Centrigrade.

Following the line of thought just expressed, groups of solders were checked carefully to determine their practical working characteristics. Observations based upon the flowing of the solders on variously shaped pieces of 22K plate, as shown in Fig. 5, gave confirmation of the desirable properties of solders of type of compositions outlined. The cylinder *A* is roughly that described in early Navy specifications for dental solders which required solders to flow the length of such a cylinder (bevel edged, annealed, pickled, and fluxed).

Two curved pieces, as shown by *B* (Fig. 5), set at a slight angle to each other serve to demonstrate the surface tension of solders, which, when fused, should pull the pieces tightly together. A small piece of solder placed on the edges of the same two pieces of plate after soldering, as in *C* (Fig. 5), should solder the two pieces throughout the upper section above the first solder joint.

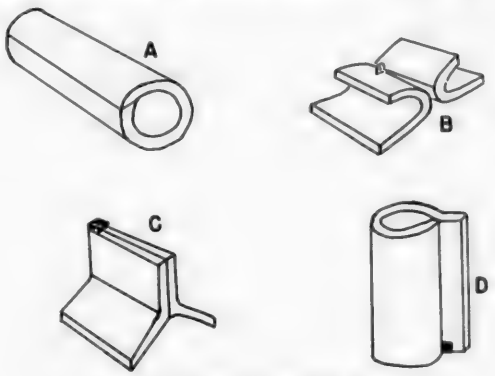


Fig. 5.—Practical soldering tests.

A very effective check on the flowing qualities of solders is provided by a cylinder as shown in *D* (Fig. 5). Solders, if properly formulated, will flow vertically along a cylinder for at least one and one-half inches when fused properly.

Other practical tests were made by flowing heavy sections of solder into small, trough-shaped sections of 22K plate to observe any tendencies to pit. Pitting, except that resulting from over heating, is so largely dependent upon the skill and care of the operator that this sort of test seemed indeterminate. Similarly, the successful use of solders in building up thick sections seems largely a question of operative skill and not an item which can be easily determined by test.

A series of specifications for solders of acceptable working qualities should include tests to assure the possession of all the desirable properties listed earlier.

Compositions alone would be unsatisfactory as a specification because of the difficulty of complete analyses. Fixed formula specifications are often con-

sidered undesirable as limiting desirable future improvements in quality. Gold and silver are, however, easy to determine by assay, and for solders of specified fineness, a silver content defined between fairly narrow limits seems one practical specification item as it will place solders in approximately the basic composition ranges which seem desirable (Area *C*, Fig. 1).

The fusible elements, which are among those difficult of analysis, influence our fusion ranges very definitely. A sufficiently large proportion of them should be present to assure the desired free flowing characteristics without having so much present as to make pitting a common occurrence. A satisfactory practical fusion test seems the best method of checking the effects of these constituents. A conventional melting range determination is not practical because of the quantity of material required for the test. Experience with the "Fusion Test—Wire Method" as defined in American Dental Association Specification No. 5 for Inlay Golds as applied to solders has not been satisfactory because of erratic results and the tendency for the determined values to fall outside (below) the fusion range.

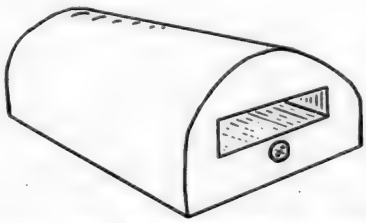


Fig. 6.—Cast silver block for fusion tests.

After considerable experimentation, a satisfactory fusion test for solders was developed. It consisted of the determinations of the temperatures at which one-sixteenth inch discs of solders .010 inch to .012 inch in thickness would: (A) first attach themselves to a fluxed plate of pure gold, (B) start to flow but still show evidence of the original shapes, and (C) flow and lose all evidence of the original form. The discs of solders were prepared by punching them from strips or pieces as received from the producer.

These tests were carried out in a silver block cast to fit the platinum-wound muffle of a porcelain fusing furnace. It contained a recess for the test samples and a round opening to accommodate a thermocouple (Fig. 6). This block was protected from outside influences by an insulating door block and temperatures at 10° C. intervals were easily obtained and maintained. Fig. 7 illustrates typical specimens representing the three points mentioned.

The establishment of a series of temperatures at which solders of various fineness should first attach themselves to plate will, if set high enough, prevent the use of too much zinc, tin, and phosphorus. A similar series of properly selected temperatures at which flow and loss of form must take place assures the necessary easy flowing characteristics. These fusion requirements in combination with specified composition limits and practical tests for satisfactory flow should insure the purchase of good quality solders under a specification.

The following requirements for dental gold solders of the grades shown were suggested as a basis for specifications covering the properties of these materials.

SUGGESTED REQUIREMENTS FOR DENTAL GOLD SOLDERS*

GOLD	SILVER		FUSION TESTS (30 SEC. AT TEMPERATURES GIVEN)		
			ATTACHES TO PLATE. HOLDS SHAPE.‡	FLOWS BUT† SHOWS EVIDENCE OF ORIGINAL SHAPE.	FLOWS AND LOSES ALL TRACE OF ORIGINAL SHAPE.§
FINENESS TO BE STAMPED ON EACH PIECE	MINIMUM (PER CENT)	MAXIMUM (PER CENT)	DEGREES F. MINIMUM	DEGREES F.	DEGREES F. MAXIMUM
.800	4	8	1440	1490	1540
.730	6	10	1440	1490	1530
.650	12	16	1430	1475	1500
.615	16	20	1410	1460	1490
.600	16	20	1400	1450	1480
.585	18	22	1390	1440	1480
.570	19	23	1390	1440	1475
.530	23	27	1380	1430	1460
.500	25	29	1380	1425	1450
.490	26	30	1380	1420	1450
.435	30	34	1380	1395	1425

*Since this paper was presented and prior to its publication, the above suggestions were first adopted as a basis for the current U. S. Navy Specification for Dental Solders and later by the U. S. Army for its purchase of these materials.

†For information only—not as a specification item.

‡When checked with a series of solders these values averaged 56 degrees Fahrenheit above the low limit of the fusion ranges.

§These values averaged 9 degrees Fahrenheit above the upper limit of the fusion range.

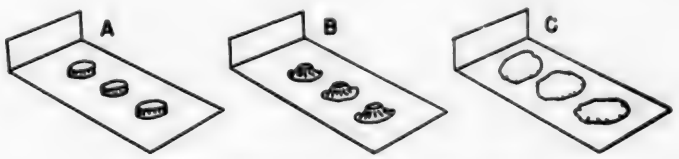


Fig. 7.—Various stages of fusion tests.

SUMMARY

1. The desirable properties of solder were studied in relation to their basic gold-silver-copper proportions.

2. Based upon consideration of fusion data by L. Sterner-Rainer,[1] basic solder compositions were indicated which should be acceptable in use.

3. Test methods were developed which made it possible to determine the fusion characteristics of dental gold solders on very small samples.

4. Series of gold solders were tested to determine the practical application of the test methods developed, and fusion data were compared with those obtained by conventional methods.

5. Suggested requirements covering the properties of gold solders ranging in fineness from .435 to .800 were outlined as a basis for purchase specifications.

REFERENCE

1. Sterner-Rainer, L.: *Ztsch. f. Metall.* **18**: 143, 1926.

THE EFFECTS OF VARIABLE FACTORS ON CRUSHING STRENGTHS OF DENTAL AMALGAMS

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DENTAL amalgams constitute a most important and very high proportion of individual tooth restorations. The physical properties of finished amalgams, upon which the restorations depend for satisfactory service, vary with the alloy formulas, manufacturing methods, mixing procedures, and condensation technics.

Previous studies of amalgams have established the effects of many variables and the American Dental Association and Federal Specifications for Dental Amalgams have established performance standards which are generally accepted.

In the earlier tentative drafts of these specifications, the physical qualities governed by detailed requirements included setting expansion, flow, and crushing strength. In later revisions the crushing strength requirements were eliminated primarily because those amalgams which met the flow requirements also met the crushing strength requirements. Notwithstanding the discontinuance of this early and rather low requirement, the crushing strength of amalgams under many conditions have been reported in dental literature.[1, 2, 3]

In considering some problems involved in the selection of testing methods for gypsum products it became apparent that some characteristics of these materials could best be determined by studying their elastic properties and crushing strengths under loads very slowly applied. As data available on the crushing strengths of amalgams gave no definite indication of the loading rates employed, it appeared of interest to observe the effects of variations in loading rates on amalgams.

An introductory series of tests to determine crushing strengths of amalgams under slow loading indicated that it would be possible to determine both elastic and plastic characteristics of these materials, and so gain some new basic information which could be related to the usefulness of these alloys in service.

After considerations of many of the variables involved in amalgam technics, series of tests were outlined which would show the effects of several individual factors. Those factors which can be controlled by the dentist were given major consideration. In recent years the increasing use of mechanical amalgamators has made their effects of interest; similarly, the effects of mechanical condensing devices are of interest to the dental profession, so these were also included.

In practice, amalgam mixes seldom contain more than twelve grains of alloy plus the required amount of mercury; and as the current amalgamators are

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not designed to handle larger mixes well, it was decided that a twelve-grain mix would be a closer approximation to the mixes used in dental practice than the large crushing strength specimen used by most previous workers. This size of mix is sufficient to make a cylindrical test specimen 4 mm. in diameter and 8 mm. long, which is the standard A.D.A. specification size for flow test specimens.

Following the selection of the specimen size, tests were outlined to show the variation of crushing strengths for amalgam when: (1) Two sizes of specimens were tested; 6 × 12 mm. and 4 × 8 mm.; (2) the loading rate (as measured by head travel of the testing machine) was varied; (3) the method of mixing was varied; (4) the method of condensation was varied; and (5) the age of the specimens when tested was varied.

Five current dental alloys listed below were selected for test.

Alloy	*Manufacturer*
Aristaloy	Baker & Company
Fine Cut	The L. D. Caulk Company
Twentieth Century	The L. D. Caulk Company
White Beauty	Lang Dental Mfg. Co.
True Dentalloy (A)	The S. S. White Dental Mfg. Co.

These include two fine cut alloys, two which would be classed as medium in particle size, and a fifth which would be considered relatively coarse cut. The following details of procedure were established:

1. Test specimens were packed in split steel molds of appropriate dimensions, clamped on the working surface of the pressure recording device developed and previously reported by Ward and Scott[1] so that it was possible to observe visually the pressure exerted by the condensing instruments.

2. Alloy samples (12 grains) and mercury were weighed on a Crandall balance. The alloy-mercury ratio was 5/8 for all specimens.

3. Mixing procedures were as follows: (A.) "Mortar mixing" indicated that the alloys were mixed in a properly conditioned S. S. White No. 9 mortar in accordance with the manufacturer's directions with the exception that trituration in the mortar was continued until results equivalent to that obtained by mulling (if specified) were obtained. (B.) The term "normal" was used to describe samples mixed in the Rose Amalgamator for time intervals which would provide characteristics in mixes as nearly as it was practically possible to duplicate those obtained by mortar mixing. (C.) Variations in the mixing time in the Rose Amalgamator to provide inadequate mixing were listed as "undermixed," and similarly extended mixing times gave "overmixed." The characteristics of the alloys tested varied markedly and Table I indicates the time intervals associated with these descriptive terms. To obtain uniformity of mixing conditions the amalgamator was operated on 110 volts A.C. Voltage was constantly checked by an indicating voltmeter and controlled by a variable transformer. Time intervals were measured by an electric timer or a stop watch.

(D.) Mercury from portions of the mixed alloys was expressed through a squeeze cloth by twisting and by finger pressure as required just prior to packing. The mixed amalgam specimens were divided into four to five portions for packing.

TABLE I

MIXING TIME IN ROSE AMALGAMATOR

ALLOY	UNDERMIX (SECONDS)	NORMAL (SECONDS)	OVERMIX (SECONDS)
1	5	8	11
2	8	13	18
3	10	15	20
4	15	20	25
5	14	20	25

4. Condensing procedures were selected as follows: (A.) "Hand" condensation indicates that samples were packed using 6- to 8-pound thrusts on a 2-mm. diameter, smooth-faced plugger stepped around the walls of the cavity and across the center after each addition of alloy until the final surface was reached. Then a 4-mm. diameter smooth-faced plugger was used and pressures increased to aid in the removal of the last excess of mercury. (B.) Specimens packed with the mechanically driven condensor indicated as "A" were prepared using a handpiece speed of 1440 r.p.m. As pressure could be applied through this instrument the terms light, medium, and heavy were used to indicate thrust pressures of 2-4 lbs., 5-6 lbs., and 7-8 lbs. Condensation was started with a plugger 2 mm. in diameter and finished with one 4 mm. in diameter. (C.) Specimens were packed with a mechanically actuated condensor indicated as "B" which provided a vibratory motion to the packing point when pressures were applied. Investigation showed that even relatively low pressures would stop the vibratory action of the instrument, and so tests were made with it by varying handpiece speeds from 1440 to 2175 and 3250 r.p.m. Pressure as applied to the instrument during these tests was approximately two pounds as shown by the manometer. Condensation was started with a point 2 mm. in diameter and finished with one 3.5 mm. in diameter. (D.) A third series of mechanically condensed specimens was prepared by using an air-actuated, impact type of condensor indicated as "C" with air valve set at notch 8 (lowest impact notch 1) and with variable compressor speeds later indicated as 3, 4, and 5, and associated with speeds of 640, 750, and 700 r.p.m. when operated on 110 volts. It was noted that the motor speed of this instrument varied greatly with voltage and motor temperatures. The voltage was controlled as described earlier for the amalgamator used, and the motor was operated at least 20 minutes before any test was made in order to increase uniformity of conditions. The straight handpiece of instrument was used with a single condensing point 3.7 mm. in diameter and pressure was applied only until the valve operated (about one to one and one-half pounds).

The variations in speed with time and voltage previously mentioned are large enough to seem worthy of consideration since they constitute a warning of possible inconsistent end results in amalgam work.

Fig. 1 illustrates the variation in the speed of the compressor used with the length of operating time. It will be noted that both the number and power of impacts produced will vary widely as the motor operates for longer intervals. Table II gives average speeds at the settings indicated after the compressor was operated 20 minutes or more, and again marked differences in performance are to be observed. (E.) Both ends of all specimens were trimmed smooth with a razor blade before removing from the steel die and while still susceptible to

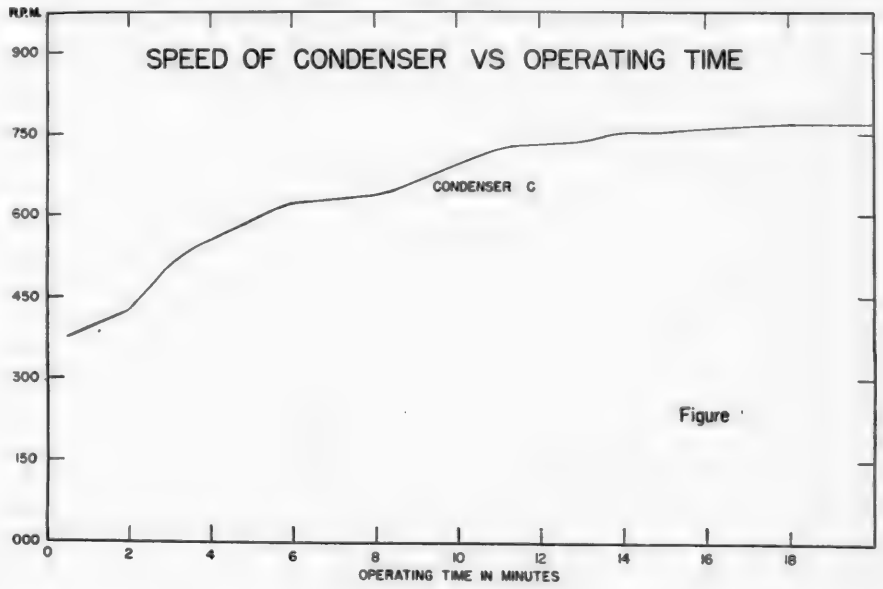

Fig. 1.

carving. Some earlier flow tests on standard alloys indicated little differences in results with specimens so prepared and those whose ends were turned in a lathe. All specimens were prepared and tested at room temperature (22° to 26° C.).

TABLE II

AVERAGE VARIATIONS IN SPEEDS OF COMPRESSOR "C" WITH VOLTAGE

SPEED SETTING	APPLIED VOLTAGE		
	105 (RPM)	110 (RPM)	115 (RPM)
3	550	640	710
4	680	750	860
5	700	770	875

TEST DATA

Specimens were prepared by various combinations of the mixing and condensing procedures outlined, and the results secured are shown in the following

tables. Unless otherwise noted the values for crushing strength represent average values for three or more specimens reported to the nearest 100 lbs. per square inch. Deviations from the averages were within plus or minus 3 per cent.

TABLE III

EFFECT OF SPECIMEN SIZE ON THE CRUSHING STRENGTH OF DENTAL AMALGAMS

Specimens seven days old—Compressed .003 inch per minute

ALLOY	MIX	CONDENSED BY:	SPECIMEN SIZE	
			4 × 8 MM. (LBS. PER SQUARE INCH)	6 × 12 MM. (LBS. PER SQUARE INCH)
2	Normal	Hand	34600	33700
3	Normal	Hand	39300	38500
4	Normal	Hand	37800	35300

TABLE IV

EFFECT OF COMPRESSION RATE ON THE CRUSHING STRENGTHS OF DENTAL AMALGAMS

Normal mix—Hand packed—4 × 8 mm. specimens—seven days old

ALLOY	COMPRESSION RATE		
	.003 INCHES PER MINUTE (LBS. PER SQUARE INCH)	.017 INCHES PER MINUTE (LBS. PER SQUARE INCH)	.024 INCHES PER MINUTE (LBS. PER SQUARE INCH)
1	39500	49800	50800
2	34600	39300	44000
3	39300	44000	48900
4	37800	45200	47100
5	37200	43000	41500

TABLE V

THE EARLY CRUSHING STRENGTHS OF DENTAL AMALGAMS

Specimens 4 × 8 mm.—Normal mix and hand condensation—Compressed .003 inch per minute

AGE OF SPECIMEN	ALLOY NO. 1		ALLOY NO. 3		ALLOY NO. 4	
	(LBS. PER SQUARE INCH)	(PER CENT 7-DAY STRENGTH)	(LBS. PER SQUARE INCH)	(PER CENT 7-DAY STRENGTH)	(LBS. PER SQUARE INCH)	(PER CENT 7-DAY STRENGTH)
Minutes						
20	2200	6	2700	7	2400	6
30	2800	7	2800	7	3600	10
40	3700	9	4000	11	4000	11
50	5300	13	4700	12	4600	12
60	5900	15	5400	14	6000	16
70	8800	22	5600	14	6600	17
90	11600	29	7400	19	7400	19
Hours						
3	18500	47	13000	33	13600	37
6	26900	69	20600	52	18800	50
9	37400	95	29200	74	27900	74
Days						
1	37100	94	32900	84	32900	87
7	39500	100	39300	100	37800	100

TABLE VI

CRUSHING STRENGTHS OF DENTAL AMALGAMS

4 × 8 mm. specimens—Seven days old—Compressed .003 inch per minute

METHOD OF MIXING	METHOD OF CONDENSING	ALLOY NO. 1 (LBS. PER SQUARE INCH)	ALLOY NO. 2 (LBS. PER SQUARE INCH)	ALLOY NO. 3 (LBS. PER SQUARE INCH)	ALLOY NO. 4 (LBS. PER SQUARE INCH)	ALLOY NO. 5 (LBS. PER SQUARE INCH)
Mortar	Hand	40500	33800	40500	38800	32600
Under mix	Hand	40100	33100	38400	39900	36000
Normal	Hand	39500	34600	39300	37800	37200
Over mix	Hand	39600	35800	40900	38400	37400
Normal	A Light	38700	29700	38000	36700	37000
Normal	A Medium	39400	33800	41400	39400	35300
Normal	A Heavy	40500	35800	40800	36700	34800
Normal	B 1	35800	30300	36600	33600	28900
Normal	B 2	39100	31600	39700	36700	34000
Normal	B 3	39500	31800	40200	37200	35200
Normal	C 3	39500	35000	39100	36300	36000
Normal	C 4	41900	35400	41900	36800	38900
Normal	C 5	42000	35600	40100	37600	38700
Mortar	A Heavy	—	32800	38300	—	—
Mortar	B 3	—	32000	37000	—	—
Mortar	C 5	—	31900	38900	—	—

The modulus of elasticity was determined on the amalgam alloys studied by placing specimens 4 mm. in diameter and 8 mm. long in the Olsen testing machine and compressing them with the head speed regulated at 0.003 inch per minute. Deformation of the specimens was measured with a sensitive dial gage (0.0001 inch) attached to the platens. Strain was calculated over the entire length of the specimen and the stress in pounds per square inch determined on the original cross-section area of the specimens. The testing procedure was to apply a 25-lb. load (approximately 1250 lbs./inch) and take a fiducial strain reading on the gage, then take subsequent readings at 50, 100, 150, 200, etc., pounds up to the maximum load while the strain was being applied at a constant rate. It was observed that a maximum load would be reached and the beam would drop, showing that the specimens were flowing or shortening at a higher rate than the load was being applied. By keeping the load on the specimens balanced, it was possible to cause them to flow for considerable time without visible fracture, producing "beer-keg" shapes of greatly increased diameter in the mid-section and with correspondingly decreased length.

It is realized that the recording of measurements of strain made by observing the change in distance between the head platens of the machine is not in accordance with the best engineering practices. It would be better to measure strain on a gauged section some distance away from the ends, but no strain gauge was available for compressive specimens having a total length of 8 mm. However, it was found that moduli computed from a stress of 5000 lbs. per square inch to 25,000 lbs. per square inch gave reproducible results. The individual values for any set of conditions used in making the specimens can be determined to within 10 per cent. The major error in the determination is caused by the use of such short gauge lengths. The average values for the moduli of elasticity are given in Table VII. They were calculated for all of the specimens whose crushing strengths were reported in Table VI.

TABLE VII

MODULUS OF ELASTICITY OF DENTAL AMALGAMS

4 × 8 mm. specimens—Seven days old—Compression rate .003 inch per minute

ALLOY	MODULUS OF ELASTICITY	
	RANGE (LBS. PER SQUARE INCH)	AVERAGE (LBS. PER SQUARE INCH)
1	1.6 to 2.1 × 10^6	1.8 × 10^6
2	1.2 to 1.7	1.4
3	1.4 to 2.0	1.7
4	1.4 to 1.8	1.6
5	1.3 to 1.9	1.7

DISCUSSION OF RESULTS

Specimen Size.—The 4 × 8-mm. specimens tested showed somewhat greater crushing strengths than the 6 × 12-mm. specimens. Under the slow load application used, it would appear that, for similarly proportioned samples, the ultimate strength recorded would vary inversely with the length of the specimens rather than being affected by relative cross sectional areas.

Compression Rate.—The head speeds used in this study were obtained by applying an auxiliary slow speed drive to an Olsen testing machine used with a 1000-pound range. The crushing strengths noted varied materially with the compression rate which, for the short samples studied, approximated closely the shortening of the specimens under load.

The .003-inch-per-minute compression rate was slow enough to make it possible to correlate head travel and load and so plot reproducible stress strain diagrams and determine with reasonable accuracy the modulus of elasticity of the specimens tested. Specimens showed a maximum resistance of stress followed by plastic flow at stresses lower than the maximum without showing fracture.

Typical stress strain curves are shown in Fig. 2. It will be noted that the point at which the elastic characteristics of the material are lost and plastic flow begins is well defined in the cross sectional stress-strain diagram (Curve *A*) and accentuated in Curve *B* by plotting stress against rate of deformation.

Higher loading rates made it impossible to correlate load and head speeds. It was evident that the load applications were so rapid that the specimens could not flow as they did at the lower speed. The higher the speed, the greater was the tendency of the specimens to crack with explosive violence. It would appear that unstated or, perhaps, undetermined loading rates might well explain the variations in crushing strengths of the same alloys as reported by various manufacturers and investigators.

The .003-inch-per-minute head speed gave average crushing strength values 15 per cent lower than those by the 0.017-inch speed and 19.5 per cent lower than the .024-inch speed. It is felt that the values obtained at the low speed would be far more indicative of the practical behavior of amalgams in dental service than those obtained from tests at higher or undefined head speeds, as they make possible the determination of the elastic characteristics of the amalgam.

Early Crushing Strengths.—The early crushing strengths of dental amalgams are of prime importance as the early development of high crushing strength serves to minimize the possibility of the destruction of amalgam restorations through the premature application of load.

The series of specimens of Alloys Nos. 1, 3, and 4 were tested at ten-minute intervals from 20 to 70 minutes, at 90 minutes, at 3, 6, and 9 hours, and at 1 day and 7 days after trituration was begun. The data secured are given in Table V in the columns, "Pounds per Square Inch" and "Per Cent of the Seven-Day Strength," the latter figure being of, perhaps, greater general usefulness, as it gives the dentist a relative idea of how long the patient should wait before masticating on the amalgam restoration.

The data on early strength are plotted against the age of the specimens in Fig. 3. The data on the percentage of seven-day strengths related to specified time intervals are shown in Fig. 4.

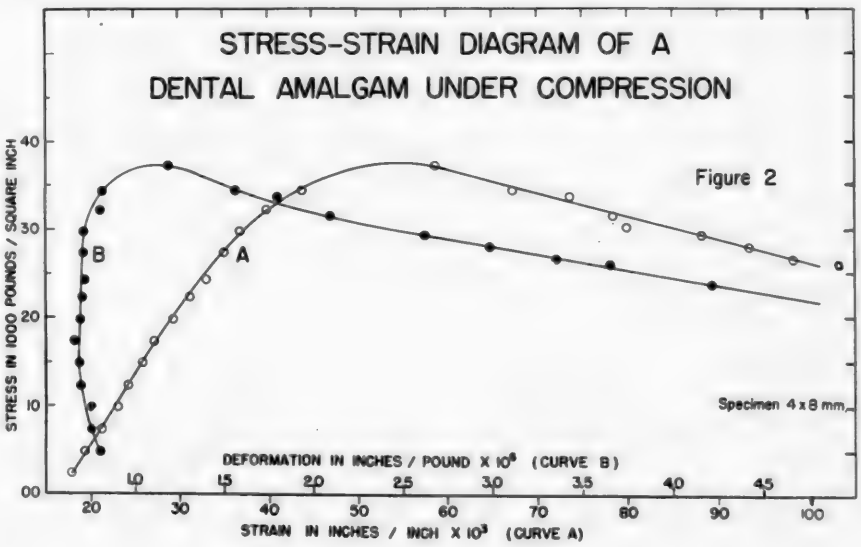

Fig. 2.

The three alloys (Nos. 1, 3, and 4) showed average increases in crushing strengths of 98, 90, and 100 pounds per square inch per minute for the first hour, setting up uniformly but slowly. In the next thirty-minute period, Alloy No. 1 showed a greater increase of strength than the others, the increases being respectively 190, 66, and 80 lbs. per square inch per minute. From ninety minutes the rates of increase in strengths were 74, 62, and 69 on the same basis. These results indicate that Alloy No. 1 would have an advantageous early strength in restorations subject to early stress.

In general, these tests for early crushing strength, though on only three alloys, appear to indicate that: (1) Mastication stresses of any sensible magnitude applied in less than one hour are dangerous to the success of amalgam

restorations as less than one fifth of the maximum strength has been attained. (2) Six hours or more should elapse between packing and the application of stress to amalgams to provide at least 50 per cent of the maximum strength. (3) After nine hours some high grade amalgams will withstand all normal stresses while others may require twenty-four hours before their crushing strengths are entirely adequate.

It should be remembered that the data reviewed were obtained at room temperatures and that amalgams under mouth conditions might logically be expected to have both a somewhat faster rate of set and a lower maximum resistance to compression stresses.

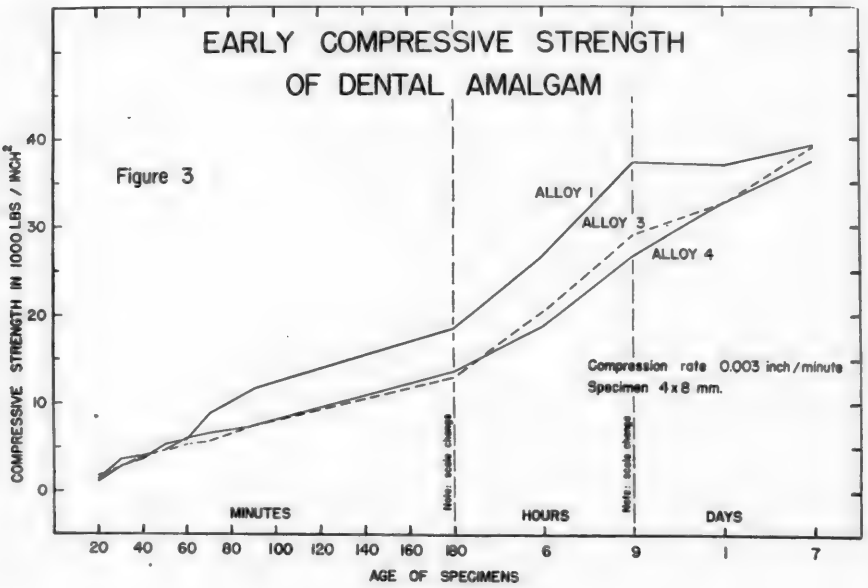

Fig. 3.

These data are also of particular interest in that they seem to correlate well with results obtained from the American Dental Association Specification No. 1 Flow Test. Flow values for the alloys studied showed that under a variety of conditions the average flow of Alloy No. 1 was 1.7 per cent, that of Alloy No. 3 was 3.2 per cent, while that of Alloy No. 4 was 5.9 per cent. The values for this last alloy were over the upper permissible limit of 4 per cent.

Alloy No. 1 showed relatively high crushing strength at the end of three hours, and this undoubtedly explains its low flow value. Alloys Nos. 3 and 4 have about equivalent strengths at the end of three hours, but the strength of Alloy No. 3 increases more rapidly during the following six hours and evidently results in a lower flow value.

Crushing tests at low speed can be carried out at intervals of approximately ten minutes. It would appear that such a test carried out at the end of a six-

hour period might well be considered as a substitute for the present specification test for flow, which has the disadvantages of requiring a separate testing instrument for each sample, and in addition, a twenty-four hour period for completion of the test. Therefore, the method suggested would definitely facilitate the testing of large numbers of alloys as several samples could be tested daily on a single machine.

Seven-day Compression Tests.—Tests were made to determine the effects of several methods of mixing and condensing amalgams on their crushing strengths.

Mechanical amalgamators are seldom, if ever, sold with adequate instructions for mixing current amalgam alloys. This renders their use in practice dangerous unless careful consideration is given to their proper control. In general it appears that users are not familiar with the wide variations in the amalgamating time of current alloys nor of the variable characteristics of the amalgamators, and so normal and undermixing and overmixing by mechanical means were included in this study.

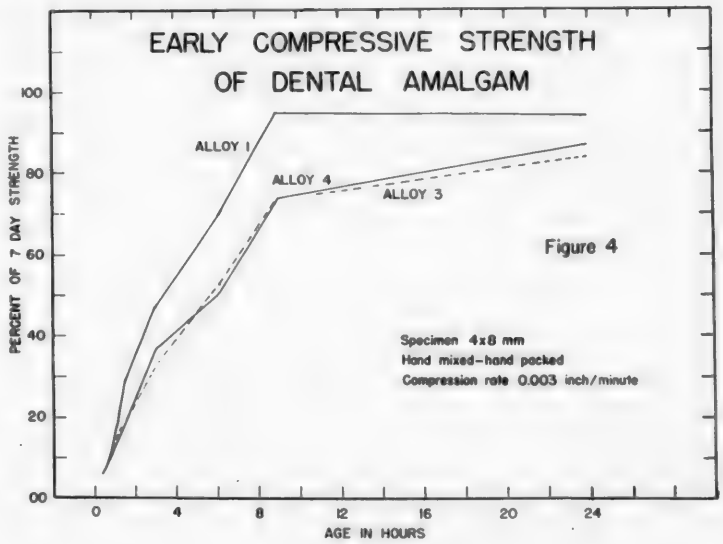

Fig. 4.

Current increased interest in mechanical condensors was the basis for the inclusion of these instruments in the investigation and those selected for study covered the available mechanical types. This type of instrument has most often been sold on the basis of generalized claims unsupported by data on its effects upon the properties of amalgams, but emphasizing the convenience of its use. There has also been a marked tendency to stress mercury removal without consideration of its possible detrimental effects upon the expansion of amalgams.

It is obvious that both types of instruments may have practical advantages for use in dental practice which laboratory tests do not take into consideration.

As the data reported here were secured strictly on a laboratory basis, no attempt has been made to evaluate the possible convenience values of either amalgamators or condensors.

Careful consideration of the data secured on the effects of variations in mixing and condensation indicates that an operator may expect no great improvements in the strength of his amalgam restorations as the result of his use of mechanical devices and that, conversely, if the instruments are improperly controlled, he may expect inferior results.

There appeared to be one set of operating conditions for each condensor which gave best results. Condensor A gave poor and erratic results at low pressures and its most consistent results when operated at intermediate pressures. Condensor B gave very poor results at slow speeds and less satisfactory results than the other instruments when operated at higher speeds although its performance showed some improvement. Most consistent results were obtained with Condensor C operated at its highest speed, but then only after sufficient warm-up time was allowed for its speed to become stabilized.

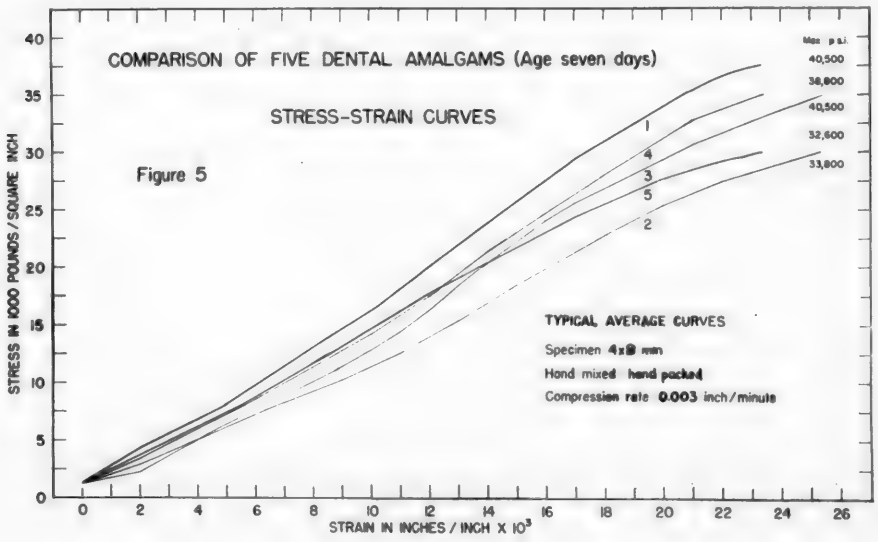

Fig. 5.

Visual observation of the hundreds of specimens included in this study warrant an opinion that mortar-mixed alloys, having been exposed to the penetration of mercury for longer periods, have somewhat better packing characteristics than mechanically triturated materials. This was borne out by an apparent greater uniformity of mortar-mixed samples as shown by the data on individual alloys, but these better packing characteristics were not directly associated with greater strengths.

Visual observation of the specimens packed by mechanical means indicate a greater tendency toward lamination with resultant possibilities of separation

of layers under light stress especially in the time intervals just following packing. This was particularly true if the specimens were dried out by the removal of mercury from each addition of alloy to a point where the surfaces showed no free mercury.

Typical average stress-strain curves for the five alloys studied are shown in Fig. 5.

Modulus of Elasticity.—The modulus of elasticity as determined by the tests described gave average values of 1.6 ± 0.2 million pounds per square inch for the five alloys tested. The method of preparation of the specimens had some effect on the value of the modulus. The higher the ultimate compressive strength, in general the higher the modulus value. This did not seem to hold for all the individual combinations of specimen preparations and possibly is connected with the amount of mercury left in the test specimen.

The fact that the modulus of elasticity of amalgam alloys is low may be an important cause of failure in service. The following list of moduli of elasticity of a few common materials will show how this property of amalgams compares with those of other materials. It should be observed that the modulus of amalgam appears very low in comparison with its ultimate compressive strength and high deformation per unit stress must be expected.

TABLE VIII

MATERIAL	MODULUS OF ELASTICITY (LBS. PER SQUARE INCH)
Copper	16.0×10^6
Dental Casting Gold	14.0
Aluminum	11.3
Gold	11.3
Magnesium	6.2
Lead	2.6
Amalgam (Dental)	1.6
Poly-Methyl Methacrylate	0.4

The modulus of elasticity is an index to the stiffness of a structural material so it would be expected that any thin edge in an amalgam filling would be very susceptible to breaking or chipping. It should be pointed out that the cavity preparation for amalgam should be such that all very thin sections which may be subjected to stress should be eliminated. Amalgam will not absorb much energy quickly without fracture, but the slow application of relatively high loads will not cause fracture although permanent deformation must be expected. It would seem desirable to investigate the relation of this property to clinical use.

The stress-strain diagrams and data obtained from the various crushing strength tests were considered to decide whether it was feasible to attempt the determination of proportional limits of amalgams. The shapes of the curves indicated that the accurate determination of this property would be difficult because of the gradually increasing rates of strain to stress. However, proportional limits were estimated by observing the stress necessary to increase the strain increment per unit of stress to 1.25 times that of its average value between 5,000 and 25,000 pounds per square inch.

The observed values were generally between 70 and 80 per cent of the ultimate compressive strengths. The inherent flow of amalgams, especially under high stress, affects the values of proportional limit, and it is felt that they do not serve as a reliable index of the serviceability of the alloys.

CONCLUSIONS

The conclusions which appear logical from consideration of the data secured in this study and of the requirements of dental amalgams in service are:

1. Crushing strengths of dental amalgams should be determined on specimens made from mixes approximating in size those commonly made by dentists. Approximately 12 grains of alloy and an appropriate amount of mercury will provide sufficient material to pack a 4 × 8-mm. test cylinder.

2. The smaller 4 × 8-mm. specimens used gave a 2 to 7 per cent increase in crushing strength over that of the 6 × 12-mm. cylinders used by previous investigators when prepared and tested under the same conditions.

3. The compression rate should be given in reporting crushing strengths of dental amalgams to permit reproduction of test data. It appears that such speeds should be standardized at approximately 0.003 inch per minute to determine both the elastic and plastic properties of amalgams.

4. The early strengths of amalgams are so low that no stress should be applied to a restoration less than an hour old, and any mastication of food on occlusal amalgam restorations less than six hours old may be expected to cause damage.

5. The compressive strengths of the five alloys studied were determined under thirteen different combinations of mixing and packing procedures. The variations in crushing strengths among these alloys were greater than those produced in the individual amalgams by varying technics.

6. The wide variation in the rates of amalgamation of the current amalgam alloys under mechanical trituration indicates that the user of such mechanical devices needs *exact directions* for mixing of the specific alloy used if he is to avoid all possibility of unsatisfactory physical properties in finished restorations.

7. Laboratory data indicated no significant improvement in the crushing strengths of amalgams as the result of mechanical rather than hand condensation. As previously mentioned, convenience may be a factor deserving of consideration from the clinical viewpoint.

8. The moduli of elasticity of the five amalgam alloys studied were within the range 1.2 to 2.1 × 10^6 pounds per square inch. The average modulus for the alloys studied was 1.6 × 10^6 pounds per square inch. This value is very low in comparison to those of the other alloys used as restorative dental materials. The clinical significance of this property warrants further study.

9. The proportional limits of the amalgams studied varied with mixing and condensing technics but fell within a range from 70 to 80 per cent of the crushing strengths observed when taken to be that stress at which the unit of deformation per unit stress exceeds the average value found in the range 5,000 to 25,000 pounds per square inch by more than 25 per cent.

10. An early compressive strength test appears to be a significant and acceptable substitute for the present specification test for flow.

11. Compression testing of amalgam in the dental materials field has resulted in a dispersion of values because of the difference in the size of specimens and the types of testing machines used. In order to confine these varying conditions of test it would seem feasible to prescribe the rate of loading, expressed in pounds per square inch per minute, to which the specimen has been subjected. Using these units for rate of loading it would not be necessary to imagine what the effect on strength would be, of the length, or the area of the specimen; or whether the specimen was loaded at so many pounds per minute or so many inches per minute. Rate of loading in pounds per square inch per minute virtually absorbs all these variables. On this basis, Table IX was computed for the results outlined in this paper using data on Alloy No. 4.

TABLE IX

SPECIMEN SIZE (MM.)	HEAD SPEED TRAVEL (INCH PER MINUTE)	LOADING RATE (LBS. PER SQUARE INCH PER MINUTE)	STRENGTH (LBS. PER SQUARE INCH)
6 × 12	0.003	9,540	35,300
4 × 8	.003	15,250	37,800
4 × 8	.017	86,300	45,200
4 × 8	.024	122,000	47,100

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SOME BIOLOGICAL CHARACTERISTICS OF THE PATHOGENIC FUNGI NAMED PARACOCCIDIOIDES BRASILIENSIS AND PARACOCCIDIOIDES CEREBRIFORMIS

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WE are interested in the agents of the South American blastomycosis because four cases were diagnosed in Uruguay by Cancela,[1, 2] and because the infection may begin as a dental apical process.[3]

South American blastomycosis has been diagnosed in Brazil, Argentina, Paraguay, Venezuela, Peru, and Uruguay. The infection is especially common in some areas of Brazil, in the states of São Pablo and Minas Geraes.

The clinical type of infection depends[4] on the way the fungus enters the body: (1) cutaneous and mucosal lesions; (2) enlargement of the lymph nodes, especially those of the neck which produce the lymphatic type; (3) visceral forms, and (4) mixed types.

The disease usually begins on the mucosal surface of the oral and nasal cavities, or on some area of the skin next to the oral or nasal orifices as papules and ulcerations. These are accompanied by regional lymph node enlargement. The infectious process invades the oral cavity insidiously, sometimes involving the larynx. The infection is more or less rapid. Death may occur within two or three months, but usually the patient lives for three or more years. Lesions are painful, granulomatous, productive, and pyogenic. The patient usually dies in cachexia.

The portal of entry in some cases is not apparent and massive cervical lymph node enlargement may be the first or essential clinical feature. It is presumed in this case that the organism enters the body through the tonsils although no lesions are seen. Bogliolo[3] studied a fatal case and found typical parasitic forms of the fungus *Paracoccidioides brasiliensis* in a granulomatous apical process on a carious molar. The fungus seems to have entered through the root canal of the carious molar.

The disease as well as the agents has been given different names by different authors. We have used the more generally accepted terminology.

The causative agent of the disease.—Three species have been isolated from cases of South American blastomycosis: *Paracoccidioides brasiliensis* (Splendore) Almeida; *Paracoccidioides cerebriformis* Moore; and *Paracoccidioides Tenuis* Moore. We could not obtain a strain of *P. tenuis* but its morphology is apparently no different from *P. brasiliensis.*

Several strains of *P. brasiliensis* have been studied, including the strain number 630 obtained from Moore. One strain obtained from Moore, *P. cerebriformis,* was assayed.

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Morphologic and biologic studies were made. The morphology was observed on glucose peptone agar (Sabouraud's), on broth agar, on Krainsky's medium, and in glucose peptone broth.

The utilization of nitrogenous compounds and different sugars was studied. For the first we used the following basal medium: potassium dihydrogen phosfate, 1 gm.; magnesium sulfate, 0.5 gm.; potassium chloride, 0.5 gm.; ferrous sulfate, 0.01 gm.; glucose, 30 gm.; glycerin, 50 gm.; agar, 20 gm.; and water, 1,000 cubic centimeters.

Eight cubic centimeters of the medium were poured into assay tubes and slanted. After solidification, 20 drops of solution containing different nitrogenous compounds were added on the surface of the agar of each slant. The solutions were: Difco bactopeptone, 10 per cent; urea, 2 per cent; asparagine, 2 per cent; sodium nitrate, 1 per cent; and ammonium sulphate, 2 per cent. To another tube (control), 20 drops of distilled water were added.

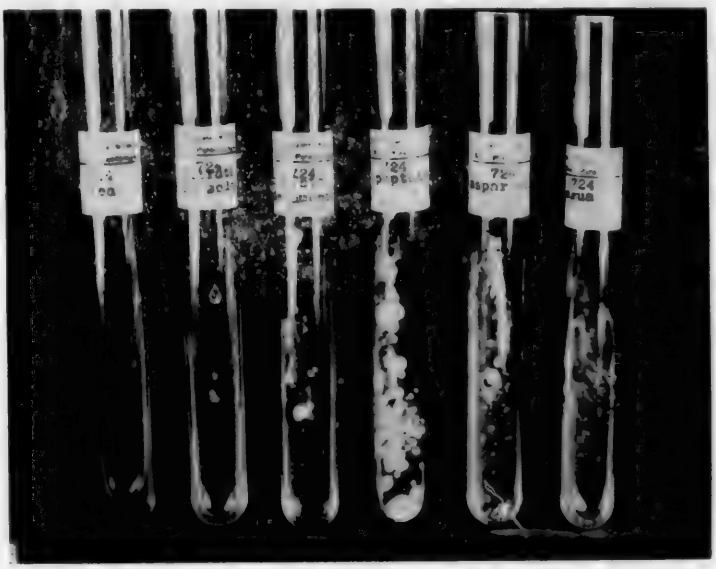

Fig. 1.—*Paracoccidioides brasiliensis* Auxanogram of nitrogenous compounds.

The utilization of different sugars (glucose, maltose, sucrose, lactose, and galactose) was studied in a basal medium similar to that of Krainsky but without the glucose. To the basal medium 20 drops of 10 per cent solution of the sugars were added. To some tubes (control) 20 drops of distilled water were added. We noted with one cross (+) scant growth in the control tube and with three crosses (+++) maximum growth.

Proteolytic action was studied on milk, gelatin, and Löffler medium. The hydrolysis of starch and the catalysis were studied by the usual methods.

Our results are shown in the tables. The differences between *P. brasiliensis* and *P. cerebriformis* appear quite clearly when the utilization of the nitrogenous compounds and sugars is considered. The optimal temperature of *P. brasiliensis*, moreover, is near 37° C. while that of *P. cerebriformis* is very low, 25° Centigrade.[5]

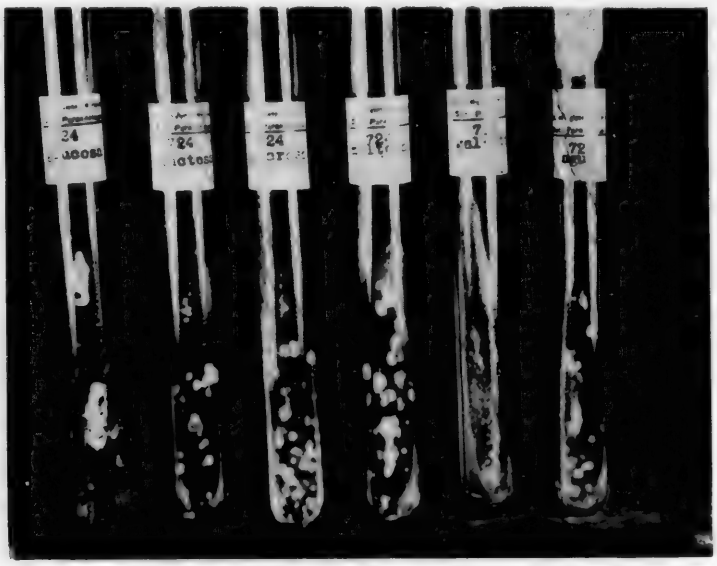

Fig. 2.—*Paracoccidioides brasiliensis* Auxanogram of sugars.

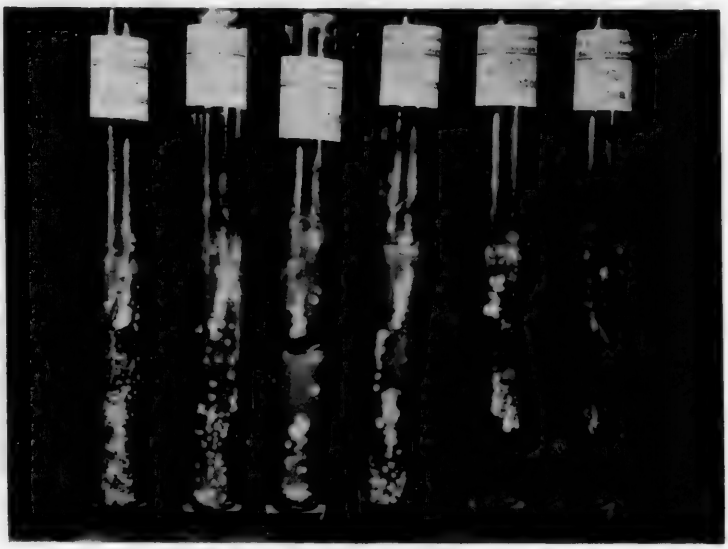

Fig. 3.—*Paracoccidioides cerebriformis* Auxanogram of sugars.

We have inoculated guinea pigs intratesticularly with both species. *P. brasiliensis* is pathogenic while *P. cerebriformis* is not pathogenic.

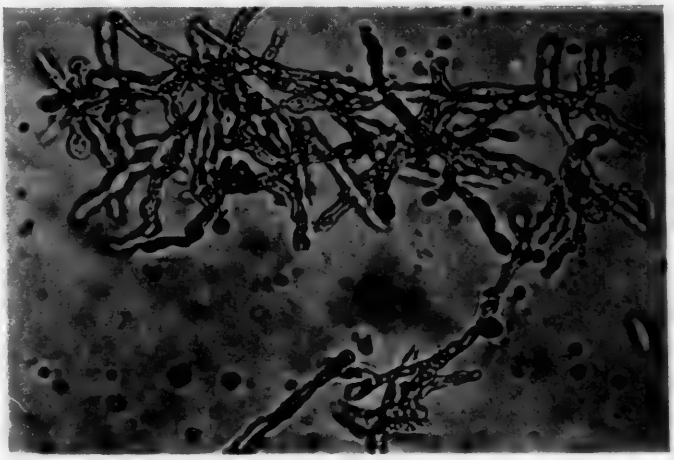

Fig. 4.—*Paracoccidioides cerebriformis.* Growth in liquid medium × 800 d.

TABLE I

P. brasiliensis	*P. cerebriformis*
MICROSCOPIC ASPECT	
Hyphae: thin, long, branched. From 1 to 1.6 microns broad.	*Hyphae:* thin or thick, globulose, branched. With characteristics similar to those of artrospored. From 2 to 7 microns broad.
Chlamydospores:	*Chlamydospores:*
Terminal: thick, round; sometimes with a hyalin capsule; at first on a spatuliform and pyriform shape. From 2.4 × 4 to 6 × 10 microns. *Intercalar:* round, from 4 to 9 microns and oval from 6 × 10 to 9 × 15 microns in diameter. *Lateral:* of very different shape.	*Terminal* or *intercalar* from 12 to 16 microns in diameter and something more in occasions.
MACROSCOPIC AND CULTURE CHARACTERISTICS	
Optimal temperature 37° C.	Optimal temperature 25° C.
On Sabouraud's medium: cottony colonies of a white color; clear medium.	*On Sabouraud's medium:* grumous sediment, clear medium.
On agar agar: small dark colony with a white aerial mycellium. There is always a deep mycellium. Circular or oval shape. Ward. Reverse of the colony: dark color.	*On agar agar:* gray colony, mat, cerebriform and of a small size. Reverse of the colony: ochreous color.
On Sabouraud's agar with glucose at 4%: good growth of dry and crusty colonies of a powdery aspect. White aerial mycellium. There also exists deep mycellium. The reverse of the colony is darker in the center than in the borders. Slight pigmentation of the medium.	*On Sabouraud's agar: with glucose at 4%:* mat colony of a white color and of a cerebriform aspect. In some occasions small colonies with aerial mycellium are seen.
On Krainsky's medium: the same aspect as when Sabouraud's agar is used but with a greater quantity of the mycellium. Dark reverse.	*On Krainsky's medium:* the same aspect as when Sabouraud's medium is used but with a poor growth.
General aspect: colonies with aerial mycellium.	*General aspect:* colonies without aerial mycellium and of a cerebriform aspect.
Velocity of growth: very slow showing some difficulty.	*Velocity of growth:* relatively easy and rapid.

TABLE II

	UREA	SODIUM NITRATE	AMMONIUM SULFATE	PEPTONE	ASPARAGINE	CONTROL	DAYS	GLUCOSE	LACTOSE	SUCROSE	MALTOSE	GALACTOSE	CONTROL	DAYS	MILK	LOFFLER MEDIUM	GELATINE	DAYS	STARCH	DAYS	CATALASA
630 *Paracoccidioides brasiliensis* Moore's strain	+	+	++	+++	++	+	30	+++	+	+	+	+	+	30	+	-	+	30/40	+	10/12	+
777 *Paracoccidioides brasiliensis* Cancela's strain	+	+	++	+++	++	+	30	+++	+	+	+	+	+	30	+	-	+	30/40	+	10/12	+
792 *Paracoccidioides brasiliensis* Arêa Lêao's strain	+	+	++	+++	++	+	30	+++	+	+	+	+	+	30	+	-	+	30/40	+	10/12	+
724 *Paracoccidioides brasiliensis* Paraguayan strain	+	+	++	+++	++	+	30	+++	+	+	+	+	+	30	+	-	+	30/40	+	10/12	+
793 *Paracoccidioides brasiliensis* Arêa Lêao's strain	+	+	++	+++	++	+	30	+++	++	+++	+++	+++	+	30	+	-	+	30/40	+	10/12	-
631 *Paracoccidioides cerebriformis* Moore's strain	+++	+++	++	+++	+++	+	30	+++	++	+++	+++	+++	+	30	+	-	+	30/40	+	10/12	-

SUMMARY

P. brasiliensis and *P. cerebriformis* are different species which may be easily distinguished by studying the utilization of some common nitrogenous compounds and sugars. The first is pathogenic for guinea pigs while the second showed no pathogenic action.

Fig. 5.—*Paracoccidioides brasiliensis.* Growth in liquid medium × 800 d. (Photographs by Mr. Marcos Santa Rosa from Seccion Fototécnica del Instituto de Higiene.)

P. tenuis could not be studied, but its small morphological differences do not allow us to distinguish it from *P. brasiliensis.*

The importance of the disease for odontologists is emphasized. Bogliolo[3] observed in one case that dental caries and apical processes may be the portal of entry of the organism into the human body, thus producing a very grave, painful, and long systemic disease.

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CARIES INCIDENCE REDUCTION BY UNSUPERVISED USE OF 27.5 PER CENT AMMONIUM THERAPY DENTIFRICE*

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EXPERIMENTING with various combinations and concentrations of ammonia-producing substances, Grove and Grove,[1] Kesel, O'Donnell, Kirch, and Wach[2] have claimed to reduce the salivary *Lactobacillus acidophilus* count of caries-susceptible individuals and Henschel[3] has claimed to reduce caries susceptibility. Wach, O'Donnell, and Hine[4] demonstrated a reduction in total titratable acidity in the mouth with quinine and urea; Stephan[5] and Muntz and Miller[6] have shown the ability of a highly concentrated urea solution (40 to 50 per cent) markedly to inhibit production of acid by microorganisms in dental plaques. Stephan and Miller[7] reported one group of eighteen patients, including controls, wherein an actual caries count was made in the mouths of the test individuals. This is admittedly too small a group to be conclusive, but it was clinical rather than laboratory evidence.

On the other hand, we have been reluctant to reveal prematurely what seemed clinical efficacy or lactobacillus reduction, since the latter especially has been so well reported elsewhere. It may be noted that some authorities dispute the consistent and dependable relationship between the lactobacillus count and caries incidence.[8] Our report was delayed, until by clinical and radiographic examination over enough years, sufficient tangible effect upon actual caries could be noted.

Grove and Grove[9, 10] employed ammonium hydroxide and spoke of "ammonia" solution and "ammonia content of the saliva." Kesel, O'Donnell, Kirch, and Wach[2] spoke of "ammonia" and "ammonia nitrogen" and showed that it is not simply alkalinity but "ammonia nitrogen" which produces the inhibitory effect. In addition to the apparent important denaturing action of urea, the ammonium ion, or $(NH_4)^+$ radical seems to be the effective agent in the buffering mechanism and for the sake of accuracy, "ammonium ion" was used in this connection by Henschel.[3]

CLINICAL DATA

The dental histories of 220 carefully observed private patients were tabulated and analyzed. The histories chosen were those of patients who had presented themselves regularly for examination and treatment over a sufficiently long period of time for the purposes of this study, and where habits of regular home

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care with regard to dentifrices were known. Otherwise the histories were unselected. Those patients to whom a 27.5 per cent active ingredient ammonium therapy dentifrice* was prescribed were briefed on the efficacy and technic of use for maximum effect in caries control. Thereafter they were checked regularly for continued use of the dentifrice but no direct effort was made to supervise home care. The children used the dentifrice in their own fashion.

The individuals, 44 per cent male and 56 per cent female, fell into four groups:

I. *Test Group*† (Table I)

One hundred consecutive cases, 36 males, 64 females, average total observation period 10.7 years; average present age 37.5; average of 3.8 teeth missing from full complement. The pre-test period averaged 7.8 years during which the usual cosmetic dentifrices were used. This autocontrol period showed an average D.M.F./yr. of 2.39. This same group, during the following average of 34.3 months, using the 27.5 per cent active ingredient ammonium therapy dentifrice, showed an average D.M.F./yr. of 1.55, a reduction of 35.2 per cent compared with the autocontrol period; a reduction of 37.5 per cent compared with the control group, test period. The D.M.F./yr. increased in 18 cases and decreased in 82 cases.

II. *Control Group*† (Table II)

Fifty consecutive cases, 16 males and 34 females, average present age 36.1; average of 5.5 teeth missing from full complement. The average over-all observation period of 12 years was arbitrarily divided into a test period of the last three years (or that period nearest to 36 months for which examination data were recorded) and a pre-test period averaging 9 years. The D.M.F./yr. was 2.48 and 2.31 respectively, an increase of 7.3 per cent of test over pre-test period. The usual cosmetic dentifrices were used throughout both periods. The D.M.F./yr. increased in 25 cases and decreased in 25 cases.

Parenthetically it is to be noted that case #N120 showing the abnormally high per cent increase of 373 for a total of only 13 D.M.F. in a total of 15.5 years, if omitted, drops the average for the group to plus 5.1 per cent, which can be considered statistically unimportant.

III. *Test Group Requiring Extensive Rehabilitation*† (Table III)

Consisted of thirty consecutive histories of patients requiring extensive operative and mouth rehabilitation, which treatment was completed. Forty-three per cent male, 57 per cent female, average present age 34.9; average of

*

Carbamide	22.5
Dibasic ammonium phosphate	5.0
Bentonite	5.0
Calcium carbonate pptd.	63.2
Sodium lauryl sulphoacetate	3.0
Saccharin sodium	.3
Volatile oils	1.0
	100.0

†All calculations in all groups were made from permanent office records based upon filed periodic full series and yearly bitewing radiographs.

TABLE 1

TEST GROUP

One hundred consecutive unselected operative cases using 27.5 per cent active ingredient ammonium dentifrice for home care.
Full mouth x-rays approximately five-year intervals. Bitewing x-rays at least yearly. Data from permanent office records.
Average age 37.5 yrs. Average total observation 10.7 years.
Average 3.8 teeth missing from full complement.
36 per cent male, 64 per cent female.
Eighty-two showed decrease D.M.F. per year during test period.
Eighteen showed increase D.M.F. per year during test period.
Over-all average *35.2 per cent decrease* D.M.F. per year of test compared with self-control period.
37.5 per cent decrease D.M.F. per year of test compared with control group, test period.

CASE	NAME	SEX	AGE	YEARS. TOTAL OBSERVATION PERIOD	MONTHS. CONTROL PERIOD. COSMETIC DENTIFRICE	D.M.F.	D.M.F. PER YEAR	MONTHS. TEST PERIOD. USING 27.5% AMMONIUM DENTIFRICE	D.M.F.	D.M.F. PER YEAR	% CHANGE
1	DM	M	30	4.0	19	10	6.32	29	12	4.96	− 21
2	BM	F	28	4.8	21	8	4.57	36	6	2.00	− 56
3	EF	F	35	9.6	89	19	2.56	26	4	1.85	− 28
4	MS	F	42	13.0	124	48	4.65	32	6	2.25	− 52
5	ML	M	45	7.3	44	13	3.54	44	1	0.27	− 93
6	SP	M	39	15.0	142	29	2.44	38	2	0.63	− 74
7	SP	F	37	11.8	103	27	3.15	39	2	0.62	− 80
8	DK	F	40	3.8	25	7	3.36	20	5	3.00	− 11
9	GP	M	44	12.1	107	16	1.80	38	9	2.84	+ 58
10	NP	F	42	12.1	107	20	2.24	38	5	1.58	− 30
11	AA	F	35	4.1	11	7	7.63	38	6	1.89	− 75
12	MK	F	20	11.7	116	42	4.35	25	23	11.04	+155
13	JG	M	50	12.3	116	27	2.79	32	6	2.25	+ 19
14	RC	F	35	9.4	84	19	2.72	29	2	0.83	− 69
15	AC	M	38	10.4	93	3	0.39	32	2	0.75	+ 92
16	MM	F	40	13.2	121	30	2.98	38	10	3.16	+ 6
17	JM	M	41	14.2	151	35	2.78	20	2	1.20	− 57
18	LK	F	20	15.8	146	33	2.71	44	1	0.27	− 90
19	BM	M	41	13.8	123	34	3.32	43	9	2.51	− 25
20	HM	F	38	13.8	126	23	2.19	40	20	6.00	+174
21	NF	M	40	6.6	53	11	2.49	26	4	1.85	− 26
22	EF	F	39	9.1	84	14	2.00	25	3	1.44	− 28
23	DS	F	44	11.6	103	15	1.75	36	4	1.34	− 24
24	HK	F	30	13.2	126	12	1.26	32	3	1.13	− 10

25	IS	M	46	11.7	102	16	1.88	39	1	0.31	− 83
26	AF	F	45	16.7	164	55	4.02	37	8	2.60	− 35
27	JF	M	50	17.6	180	25	1.67	31	5	1.93	+ 16
28	HB	M	38	16.4	156	12	0.92	41	3	0.88	− 5
29	CB	F	37	11.4	96	70	8.75	41	5	1.47	− 83
30	GC	F	40	10.4	88	16	2.18	37	9	2.92	+ 34
31	LB	F	15	7.8	52	12	2.77	41	5	1.47	− 47
32	HS	F	40	5.2	28	7	3.00	34	2	0.71	− 76
33	JS	M	45	4.3	18	9	6.00	34	1	0.35	− 94
34	MH	F	45	15.2	146	28	2.30	37	6	1.95	− 15
35	NH	M	48	15.6	150	26	2.08	37	7	2.27	+ 10
36	CM	F	40	16.1	155	19	1.47	38	3	0.95	− 35
37	CM	F	12	6.4	39	4	1.23	38	3	0.95	− 23
38	HM	M	42	6.2	35	14	4.80	39	2	0.62	− 87
39	LJ	F	35	6.3	53	8	1.81	23	0	0.00	−100
40	JH	F	39	4.3	14	5	4.29	38	0	0.00	−100
41	FW	M	17	8.2	76	14	2.21	22	4	2.18	− 1
42	JW	M	25	14.5	148	34	2.76	26	8	3.69	+ 34
43	LC	F	35	6.9	56	27	5.78	27	4	1.78	− 69
44	PK	M	55	6.9	60	6	1.20	23	3	1.57	+ 31
45	FB	F	19	9.1	64	29	5.44	45	17	4.53	− 17
46	LD	M	35	6.6	49	11	2.69	30	4	1.60	− 41
47	HP	F	36	13.7	127	55	5.20	37	11	3.56	− 32
48	PS	F	40	11.3	91	40	5.28	45	19	5.07	− 4
49	RW	F	34	16.4	165	20	1.45	31	0	0.00	−100
50	MW	M	35	16.4	165	66	4.80	31	13	5.03	+ 5
51	LD	M	41	12.1	108	27	3.11	37	6	1.95	− 37
52	MF	F	35	9.8	81	12	1.78	37	1	0.32	− 82
53	LF	M	46	8.9	72	22	3.67	35	6	2.06	− 44
54	RF	F	35	9.2	73	10	1.65	37	0	0.00	−100
55	LK	F	52	7.2	63	6	1.14	24	3	1.50	+ 31
56	LM	F	36	4.7	18	4	2.67	39	0	0.00	−100
57	MS	M	40	10.4	80	30	4.50	45	9	2.40	− 47
58	BS	F	39	6.9	35	18	6.17	41	7	2.05	− 67
59	FC	F	40	6.6	50	11	2.64	29	3	1.24	− 53
60	SK	F	23	10.3	78	46	7.08	45	0	0.00	−100
61	BJ	F	34	5.8	42	16	4.57	28	6	2.57	− 44
62	DS	F	20	9.2	80	5	0.75	30	0	0.00	−100
63	LS	M	50	12.2	112	3	0.32	35	0	0.00	−100
64	RS	F	48	8.5	71	7	1.18	31	0	0.00	−100
65	LM	F	42	16.8	182	20	1.32	20	6	3.60	+171
66	SM	M	48	16.9	171	11	0.77	32	1	0.38	− 51
67	MF	F	40	14.7	142	34	2.87	34	4	1.41	− 51
68	CM	F	17	9.1	76	17	2.69	33	5	1.82	− 32

TABLE I—CONT'D

CASE	NAME	SEX	AGE	YEARS. TOTAL OBSERVATION PERIOD	MONTHS. CONTROL PERIOD. COSMETIC DENTIFRICE	D.M.F.	D.M.F. PER YEAR	MONTHS. TEST PERIOD. USING 27.5% AMMONIUM DENTIFRICE	D.M.F.	D.M.F. PER YEAR	% CHANGE
69	CH	M	41	19.0	180	10	0.67	47	2	0.51	- 24
70	SH	F	40	19.0	180	7	0.47	47	0	0.00	-100
71	CK	M	50	11.7	104	10	1.15	36	2	0.67	- 42
72	LD	F	41	18.0	179	16	1.07	36	3	1.00	- 7
73	RR	F	40	7.2	50	10	2.40	36	4	1.33	- 45
74	RR	F	38	6.8	48	9	2.25	34	0	0.00	-100
75	JA	F	36	6.3	42	9	2.57	34	6	2.12	- 18
76	LA	M	42	18.5	190	21	1.33	32	1	0.38	- 71
77	SR	M	46	6.4	41	1	0.29	36	0	0.00	-100
78	LR	F	40	7.3	53	10	2.26	34	5	1.77	- 22
79	IK	M	38	18.3	194	18	1.11	26	0	0.00	-100
80	MO	M	44	7.4	68	3	0.53	21	1	0.57	+ 8
81	RO	F	10	4.5	24	2	1.00	30	2	0.80	- 20
82	DO	F	15	8.8	75	10	1.60	31	0	0.00	-100
83	FO	F	40	9.3	81	8	1.19	31	1	0.39	- 76
84	BL	M	48	15.5	147	9	0.74	39	1	0.31	- 58
85	FL	F	42	11.4	102	11	1.29	35	2	0.69	- 47
86	BK	F	48	11.4	104	19	2.19	33	5	1.82	- 17
87	HF	M	44	7.4	57	10	2.11	32	5	1.88	- 11
88	ES	F	17	7.3	58	15	3.10	29	5	2.07	- 33
89	PH	M	38	13.6	120	58	5.80	43	6	1.67	- 71
90	EH	F	36	13.6	120	6	0.60	43	5	1.40	+133
91	IA	F	29	6.8	41	10	2.93	40	5	1.50	- 49
92	RH	F	37	7.5	59	11	2.24	31	6	2.32	+ 4
93	EA	F	35	6.3	36	17	5.66	40	5	1.50	- 74
94	SO	F	39	18.3	187	25	1.60	31	2	0.77	- 52
95	MO	M	41	16.8	171	17	1.19	31	3	1.16	- 3
96	EW	F	55	16.1	154	29	2.26	39	1	0.31	- 86
97	RB	F	40	11.7	116	22	2.28	25	7	3.36	+ 47
98	IL	F	36	5.6	31	7	2.71	36	3	1.00	- 63
99	EK	F	37	12.0	105	28	3.20	39	7	2.16	- 33
100	GK	M	43	4.5	16	1	0.75	38	0	0.00	-100
Final percentage is weighted average		M. 36% F. 64%	37.5 Age	10.7 Years	93.8 Months	18.7 D.M.F.	2.39 D.M.F./Yr.	34.3 Months	4.4 D.M.F.	1.55 D.M.F./Yr.	- 35.2%
									Totals:	- 82 + 18	

5.8 teeth missing from full complement. In the period averaging 27.3 months subsequent to completion of treatment during which a 27.5 per cent active ingredient ammonium therapy dentifrice was used, the D.M.F./yr. was 1.00 (including silicate replacements), well below the 1.55 of the test group, test period. This C.M.R. group was not incorporated into the test group of 100 since for obvious reasons it would have unduly influenced the final figure.

IV. *Test Group of Children** (Table III)

Forty consecutive cases of children, average age 9.8 years; 60 per cent male, 40 per cent female; using the 27.5 per cent ammonium therapy dentifrice in their own fashion for an average of 27.7 months. The D.M.F./yr. is 1.72. (This figure includes deciduous teeth, but does not include loss or extraction for causes other than caries.) Although not calculated against a similar control group, it is not abnormal, being slightly above the 1.55 of the test group and well below the 2.48 of the control group.

DISCUSSION

Henschel[3] originally experimented with formulas with as high a concentration as 66 per cent carbamide. The present formula was evolved as having the highest content of active ingredients which under average home use caused no irritation. It is believed the dentifrice acts by virtue of the protein denaturing action of its high urea content[6] which combined with the ammonium phosphate produces a strong buffering action at the site of acid production.[5] High concentrations of urea were shown to be necessary for greatest effectiveness, as well as for rapid penetration of the intact dental plaque.

In averaging the figures, equal weight was given to each D.M.F. by averaging the total time and total D.M.F. and establishing the ratio D.M.F./yr. from these figures. On the other hand, if the total of individual D.M.F./yr. is averaged, thus giving more equal weight to each patient, the results will vary from the original as noted in Table IV. With this analysis, despite a drop of 2.48 per cent in the control group, test period, the caries incidence reduction of the test group becomes 41.9 per cent; it becomes 41.2 per cent compared with the autocontrol period.

A drop in caries-incidence experience would normally be expected in the control group as the patients grew older and is easier of explanation than the rise of 7.3 per cent in the original totals. It is felt, however, that the more conservative figures of 35.2 per cent and 37.5 per cent more accurately reflect the true analysis of the caries experience of this group of patients.

This report may be considered the first of a continued study of these same cases. It is hoped that further data on these patients will be available for report in the due course of time.

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*All calculations in all groups were made from permanent office records based upon filed periodic full series and yearly bitewing radiographs.

TABLE II

CONTROL GROUP

Fifty consecutive unselected operative cases using cosmetic (nontherapeutic) dentifrices for home care.
Full mouth x-rays approximately five-year intervals. Bitewing x-rays at least yearly. Data from permanent office records.
Average age 36.1. Average total observation period 12 yrs.
Average 5.5 teeth missing from full complement.
32 per cent male, 68 per cent female.
Twenty-five showed decrease D.M.F. per year during test period.
Twenty-five showed increase D.M.F. per year during test period.
Over-all average 7.3 per cent increase D.M.F. per year of test over control period.

CASE	NAME	SEX	AGE	YEARS. TOTAL OBSERVATION PERIOD	MONTHS. PRE-TEST PERIOD COSMETIC DENTIFRICE	D.M.F.	D.M.F. PER YEAR	MONTHS. TEST PERIOD. COSMETIC DENTIFRICE	D.M.F.	D.M.F. PER YEAR	% CHANGE
N101	FG	F	40	10.8	97	17	2.10	33	12	4.35	+101
N102	AF	F	32	6.7	39	10	3.08	41	6	1.76	- 43
N103	LF	F	35	8.7	72	4	0.67	33	5	1.82	+171
N104	EG	F	13	6.5	45	12	3.20	33	5	1.82	- 43
N105	MB	M	41	18.8	193	35	2.18	32	10	3.75	+ 72
N106	RF	F	55	16.9	154	18	1.40	49	6	1.47	+ 5
N107	MS	M	38	7.8	62	11	2.13	31	6	2.32	+ 9
N108	MS	F	35	5.2	34	23	8.11	28	13	5.56	- 31
N109	PH	F	33	7.1	52	17	3.92	33	6	2.18	- 45
N110	JK	F	30	17.6	180	33	2.20	31	8	3.10	+ 41
N111	CH	M	38	13.6	126	26	2.48	37	8	2.60	+ 5
N112	GK	F	45	18.4	184	29	1.89	36	6	2.00	+ 6
N113	VL	F	30	11.6	101	22	2.62	38	6	1.90	- 28
N114	PB	M	70	18.8	191	16	1.00	34	6	2.12	+112
N115	PB	F	60	18.8	191	18	1.13	34	7	2.47	+118
N116	MS	M	47	18.5	171	10	0.70	51	1	0.24	- 66
N117	FS	F	45	18.4	177	27	1.83	44	1	0.27	- 85
N118	BS	F	16	10.4	85	12	1.69	40	10	3.00	+ 77
N119	SI	F	25	9.0	72	42	7.00	36	9	3.00	- 57
N120	BS	F	50	15.5	149	6	0.48	37	7	2.27	+373

N121	SW	M	21	4.3	25	8	3.84	27	9	4.00	+ 4
N122	RV	M	19	12.0	102	13	1.53	42	6	1.71	+ 12
N123	PG	M	60	15.0	132	13	1.18	48	3	0.75	- 37
N124	MG	F	50	15.3	137	26	2.28	47	8	2.04	- 11
N125	NG	M	53	8.5	63	8	1.53	39	4	1.23	- 20
N126	SL	F	38	16.6	159	5	0.38	40	2	0.60	+ 58
N127	SK	F	16	4.5	33	7	2.55	21	5	2.86	+ 12
N128	AP	F	32	16.0	154	16	1.25	38	3	0.95	- 24
N129	AC	F	35	12.0	109	19	2.09	35	5	1.72	- 18
N130	BF	F	36	18.4	182	23	1.52	39	11	3.39	+123
N131	MN	F	38	6.9	49	2	0.49	36	3	1.00	+104
N132	HS	F	41	7.3	56	9	1.93	32	6	2.25	+ 17
N133	HK	F	43	16.2	147	33	2.69	47	5	1.28	- 52
N134	EM	F	29	8.1	58	18	3.72	40	9	2.70	- 28
N135	IS	F	42	8.3	48	6	1.50	52	7	1.62	+ 8
N136	RK	F	17	10.8	96	41	5.13	34	31	10.93	+113
N137	VE	F	18	12.3	120	32	3.20	28	6	2.57	- 20
N138	PY	F	21	14.0	132	9	0.82	36	2	0.67	- 18
N139	LP	M	17	8.7	72	23	3.83	32	16	6.00	+ 56
N140	HS	M	32	15.2	156	29	2.23	27	3	1.33	- 40
N141	RS	M	30	16.2	156	33	2 54	38	5	1.58	- 38
N142	FS	F	33	7.0	60	27	5.40	24	4	2.00	- 63
N143	EV	M	52	15.1	120	31	3.10	62	13	2.52	- 19
N144	WF	M	38	13.5	120	28	2.80	42	10	2.86	+ 2
N145	JC	F	16	7.3	66	30	5.45	21	16	9.12	+ 67
N146	BT	F	50	7.8	60	27	5.40	34	15	5.29	- 2
N147	RP	F	25	7.0	54	36	8.00	30	9	3.60	- 55
N148	NH	M	38	8.3	48	8	2.00	51	10	2.35	+ 18
N149	EK	M	45	13.0	132	40	3.63	24	6	3.00	- 17
N150	MG	F	45	12.5	120	43	4.30	30	7	2.80	- 35
Final percentage is weighted average		M. 32% F. 68%	36.1 Age	12.0 Years	107 Months	20.6 D.M.F.	2.31 D.M.F./Yr.	36.5 Months	7.54 D.M.F.	2.48 D.M.F./Yr.	+ 7.3%
									Totals:	+ 25 - 25	

TABLE III

COMPARATIVE AVERAGES

GROUP	TOTAL PATIENTS	SEX %	AGE	TEETH MISSING	YEARS TOTAL OBSERVATION	PRE-TEST PERIOD			TEST PERIOD			% CHANGE D.M.F/YR.	NO. OF PATIENTS SHOWING REDUCED D.M.F/YR.
						MONTHS	D.M.F.	D.M.F. PER YEAR	MONTHS	D.M.F.	D.M.F. PER YEAR		
I. *Test*	100	M. 36 F. 64	37.5	3.8	10.7	93.8	18.7	2.39	(Using 27.5% Ammonium Dentifrice) 34.3	4.4	1.55	−35.2%* −37.5%†	82
II. *Control*	50	M. 32 F. 68	36.1	5.5	12.0	107.0	20.6	2.31	(Using Cosmetic Dentifrice) 36.5	7.54	2.48	+ 7.3%	25
III. *Test completed mouth rehabilitation*	30	M. 43 F. 57	34.9	5.8	–	–	–	–	(Using 27.5% Ammonium Dentifrice) 27.3	2.27	1.00		
IV. *Test children*	40	M. 60 F. 40	9.8	–	–	–	–	–	(Using 27.5% Ammonium Dentifrice) 27.7	3.98	1.72		

*Compared with autocontrol period.
†Compared with control group, test period.

TABLE IV

GROUP	PRE-TEST PERIOD D.M.F./YR.	TEST PERIOD D.M.F./YR.	% CHANGE D.M.F./YR.
Test	2.69	1.58	–41.2* –41.9†
Control	2.72	2.65	–2.48

*Compared with autocontrol period.
†Compared with control group, test period.

SUMMARY AND CONCLUSION

The caries-incidence experience of 220 private patients is reported in relation to the use of cosmetic and therapeutic dentifrices with unsupervised average home care. The test group of 100 cases used cosmetic dentifrices an average of 7.8 years, average D.M.F./yr. 2.39. Using a 27.5 per cent active ingredient ammonium therapy dentifrice an average of 34.3 months, average D.M.F./yr. 1.55. Control group of 50 used cosmetic dentifrices throughout observation period of 12 years; pre-test period average D.M.F./yr. 2.31, test period 2.48 (Table III).

An average caries-incidence reduction of 37.5 per cent is noted for the test group over the control group during the test period; 35.2 per cent reduction of the test group, test period over the autocontrol period, same group. A reduction was manifest in 82 per cent of the test patients.

By weighting the figures to give more equal consideration to each patient instead of each D.M.F., the figures are 41.9 per cent and 41.2 per cent respectively (Table IV). We would tend to minimize even the former figures because, as a matter of fact, some proportion of the apparent caries-incidence reduction may be attributed to enthusiastic oral hygiene heightened by the knowledge of the possible therapeutic benefit of the dentifrice.

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THE EFFECT OF PENICILLIN ON DENTAL CARIES IN RATS FED ON A COARSE CORN DIET

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THIS experiment was undertaken in conjunction with studies at this University on the effect of penicillin on human caries. Rat caries is sufficiently similar to this disease in man so that, experimentally, some light can be focused on the problem.[1]

Previous information as to the effect of this antibiotic has been reported.[2] McClure and Hewitt used rats on a caries-producing diet, and added 75 units of penicillin per gram of diet to the experimental group. The drinking water also contained 75 units per milliliter. It was felt that this amount of penicillin was maximal; therefore the present study was undertaken to determine the minimal amount of penicillin in water which could reduce caries incidence in the presence of a caries-producing diet alone, as well as with sucrose in the drinking water.

METHOD OF INVESTIGATION

Hoppert, Webber, and Canniff[3] demonstrated that coarse corn particles induce caries-like lesions in rat molars. This method was employed. After pulverization of whole corn, a sifting of a sample lot on standard screens gave: 60 per cent on a 20-mesh screen, 25 per cent on a 40-mesh screen, 15 per cent on or through a 60-mesh screen. This is somewhat coarser than the original H-W-C Diet. All groups in the experiment were fed the Hoppert diet ad libitum and unvaried as to content.

The method used in giving penicillin supplements was solely by way of the drinking water. This assured bathing of the molars in a fixed level of penicillin. Stock solutions were kept refrigerated at 5° Centigrade. Dilutions from these stock solutions for the drinking water were made daily or every two days. There was little question as to the stability of the penicillin solutions refrigerated at 5° C. for 10 days[4] or those standing at room temperature for 24 hours.[5] However, the potency of the nonrefrigerated solutions was assayed in our laboratory against *Lactobacillus acidophilus* and *Micrococcus pyogenes* var. *aureus* and were found to be satisfactory.

Since it has been shown that sucrose supplements promote the production of larger carious lesions in white rat molars, although it does not necessarily change the incidence of caries,[6, 7] the protective quality of penicillin was also tested in the presence of this fermentable carbohydrate. A 1.5 per cent aqueous solution was used.

The production time of caries initiated by this method has been studied by various groups. Lilly and Wiley[8] found caries in 66 per cent of their rats in

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100 days, and 100 per cent in 125 days. Kearney, Sognnaes, and Bibby[9] found that caries did not occur until about 12 weeks on the diet. Rats in this study were fed the diet for approximately 110 days, which was considered ample for the production of caries.

Twenty- to thirty-day-old female rats, whose molars were just in the process of erupting, were obtained from the stock colony maintained at Mount Sinai Hospital, Cleveland. They were divided at random into various groups as to drinking water as follows:

A—Water, B—Penicillin 1 μ/ml. in water, C—Penicillin 2 μ/ml. in water, D—Penicillin 5 μ/ml. in water, E—Sucrose 1.5 per cent in water, F—Penicillin 2 μ/ml. in 1.5 per cent aqueous sucrose solution, and G—Penicillin 5 μ/ml. in 1.5 per cent aqueous sucrose solution.

All rats were individually ear-marked for identification purposes, but each group was kept in a single cage. Daily water consumption records were kept for the groups, and weekly weight records were kept for the individual rats.

At the completion of the study, before each rat was sacrificed, a qualitative estimate of oral *L. acidophilus* was obtained by swabbing the teeth and mucosa with a small sterile cotton swab and immediately plating this material on tomato juice agar media.

Most investigations of caries in rats have reported lesions in unsectioned teeth as they are observed under low power magnification. A 20× binocular microscope was used in this study. Three types of caries-like lesions were found: (A) Fissure caries: described by Rosebury, Karshan, and Foley[10] as a penetration through the enamel and caries in the dentin of the sulci, with consequent loss of tissue. It can be regarded as analogous with the fissure caries of man. (B) Fractures: these lesions may be pathological or not, and in their advanced stages in some cases were indistinguishable from advanced fissure caries. (C) Interproximal caries: an infrequent finding of the type observed by Klein and McCollum,[11] consisting of a breaking down of the enamel substance at the approximating surfaces of the molars.

In determining, under low power magnification, just what is or is not caries, there exists great latitude. A sulcus may be fairly light yellow, yet show a large loss of tissue; it may be dark brown, yet show no loss of tissue. A fracture must be examined carefully to discover the presence, absence, or extent of the carious lesion. Arbitrarily, to prevent extreme error in observation, the standard for fissure caries in this study was the absence of a distinct sharp line or groove at the bottom of the sulcus, indicating a loss of tissue. All teeth were examined for caries without the identifiable code. The reported findings are a combination of the authors' examinations.

There are two phases of caries which require separate analysis. One of these relates to the susceptibility of an area to the initiation of the carious process; the other is the rate at which caries progresses after initiation. For susceptibility to initiation, the data are frequency of carious attack without consideration as to extent. For rate of progress it is necessary to compare size of cavities. In order to evaluate rate of progress, three values were assigned to the extent of caries: 1—slight loss of dentin, 2—great loss of dentin, and

3—pulpal exposure. The "weighed value" represents the addition of the values assigned to the carious teeth divided by the total number of teeth exposed to caries.

Harrison[12] studied the changes of flora on the surfaces of rat molars during the development of caries. The reaction of the oral flora to caries susceptibility and to the effects of inhibitors, such as fluorides and iodoacetic acid, suggested, first, that the lactobacilli are associated with experimental caries as etiologic agents, not as secondary invaders which are increased because the lesions provide a more favorable environment; and second, experimental caries is prevented by inhibition of the growth and activity of these organisms. The relative presence or absence of these organisms serves as a useful indicator of caries activity. Kesel, O'Donnell, Kirch, and Wach[13] stated that the bacteria found in the human oral flora which seems to be responsible for much of the ammonia nitrogen production is *Bacillus lactis aerogenes*. This gram-negative bacillus produces ammonia which has a limiting effect on aciduric bacteria. *B. lactis aerogenes* grows well on tomato juice agar media, and the presence of the organisms was recorded if found on a plate.

EXPERIMENTAL RESULTS

The consumption of water by all groups was well within the biological limits or normality. Those in the sucrose groups had a larger daily consumption as compared to the water groups. This is explained by the work of Richter[14] who pointed out the fondness of rats for sugar solutions. Penicillin fed to white rats enhanced growth in some cases. In no case did it hinder normal incremental growth.

Penicillin in water up to five units per ml. did not reduce markedly either frequency of attack or progress of caries (Table I). However, under conditions simulating those of the human oral cavity, i.e., with a daily intake of sugar, there was a definite and constant reduction of frequency and rate of progress with each increment of penicillin to the water supply (Table II).

TABLE I

EFFECT OF WATER CONTAINING PENICILLIN ON THE CARIES ACTIVITY OF RATS

	CONTROL WATER		PENICILLIN 1 UNIT		PENICILLIN 2 UNITS		PENICILLIN 5 UNITS	
	NO.	%	NO.	%	NO.	%	NO.	%
Rats	27		15		22		16	
Rats carious	19	70.00	10	66.00	15	68.00	8	50.00
Carious teeth	36	11.10	26	14.40	27	10.20	24	12.50
Caries weight	50	0.154	38	0.155	35	0.132	26	0.135

TABLE II

EFFECT OF PENICILLIN CONTAINING WATER ON THE CARIES ACTIVITY OF RATS RECEIVING SUCROSE SUPPLEMENT

	CONTROL SUCROSE		1.5% SUCROSE AND PENICILLIN—2U.		1.5% SUCROSE AND PENICILLIN—5U.	
	NO.	%	NO.	%	NO.	%
Rats	10		20		16	
Rats carious	10	100.00	18	90.00	11	68.00
Carious teeth	27	22.50	41	16.60	18	9.30
Caries weight	40	0.333	70	0.292	32	0.166

As the increment of penicillin increased, *L. acidophilus* present in the oral flora decreased. In the presence of sugar plus penicillin, *B. lactis aerogenes* replaced the lactobacillus as the predominant organism in the rat's mouth (Fig. 1).

SUMMARY

An experiment was conducted with rats fed a Hoppert diet, some of which received plain water, others the same diet with the varying amounts of penicillin in the water, and some with sucrose and penicillin added to the water. The rats were fed for 110 days. The control rats showed that 70 per cent of

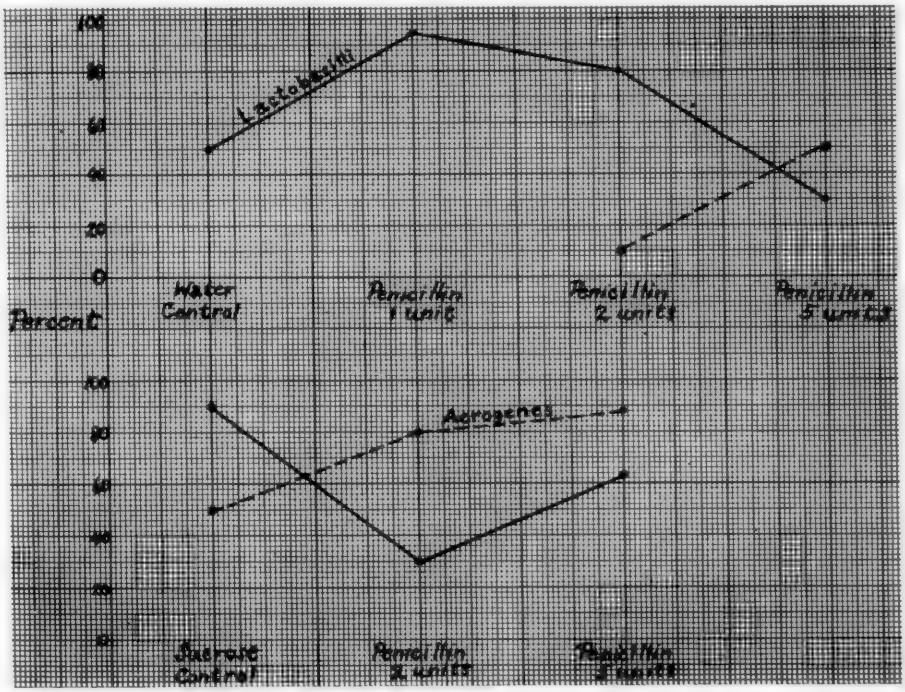

Fig. 1.—Occurrence of lactobacilli and *B. lactis aerogenes* in rat mouth flora.

them developed dental caries, and in the rats receiving 5 units of penicillin per c.c. of water supply, 50 per cent had dental caries. When a weighted value was given to the carious lesions on the basis of the amount of tooth structure which was destroyed by values of 1 for incipient caries, 2 for deep caries of the dentin, and 3 for caries which involved pulp exposures, the caries control rats had a weighted value of .154 and the rats receiving 5 units of penicillin a weighted value of .135. The rats receiving sucrose showed quite a different situation. One hundred per cent of those receiving additions of 1.5 per cent of sucrose to the water had dental caries. Of the rats which received additions of 5 units of penicillin per c.c. and 1.5 per cent of sucrose to the water, 68 per cent had carious teeth. When similar weighted values were given to the carious lesions,

the rats receiving sucrose additions had a caries weight of .353 and those with penicillin and sucrose a caries weight of .166. The percentage of carious teeth was also markedly reduced from 22.5 per cent in the rats receiving sucrose to 9.3 per cent in the rats receiving sucrose and penicillin.

Ingestion of penicillin in water by white rats reduced the occurrence of *L. acidophilus*. In those rats receiving penicillin-sucrose supplements in the water, a marked shift of the oral flora to that of *B. lactis aerogenes* occurred.

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PENICILLIN DENTIFRICE AND DENTAL CARIES EXPERIENCE IN CHILDREN

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INTRODUCTION

RECENT investigations have demonstrated that induced caries in rats can be prevented by additions of penicillin to their food and water supply,[1] and that a marked reduction in the frequency and progress of caries can be accomplished by supplementing only the water supply of rats with minimal amounts (5 units per ml.) of penicillin.[2] The experimental brushing of hamsters' teeth with a dentifrice containing penicillin has been reported as highly effective in lowering the number of carious lesions which appeared in these animals.[3] Nowhere in the literature can be found a report of the clinical use of an antibiotic for its possible influence on human caries experience.

This study was undertaken to determine what benefits, if any, might be obtained from the use of a penicillin dentifrice by children for one year under conditions comparable to the ordinary nonregimented habits of oral hygiene.

METHOD OF INVESTIGATION

Two hundred forty institutionalized boys, aged eight to fifteen years inclusive, were examined clinically with mouth mirrors and exploring tines for existent dental caries, dental restorations, and missing teeth. Only one of the authors made the examinations. Posterior interproximal radiographs were made and their interpretations were entered on individual charts. Information concerning previous residences was obtained to establish possible exposure to water supplies with known fluoride content.

In order to describe more adequately the initial extent and, later, the progress of carious lesions, a "weighted value" was recorded as follows: A weight of 1—caries of enamel; a weight of 2—shallow caries of dentin (less than half the distance from the dentino-enamel junction to the dental pulp); a weight of 3—deep caries of dentin (more than half the distance from the dentino-enamel junction to the dental pulp without apparent pulp involvement); and a weight of 4—apparent exposure of the dental pulp.

The number of carious surfaces present in each group existent at the beginning of this study was to be compared with the number found at the final examination. Surfaces found restored at the final examination were given the same caries weighted value as that which existed in the first examination. If no caries was recorded on a surface which had been restored during the year,

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an arbitrary weighted value of 2 was given. Teeth lost by caries were given the value of 4. For example, a molar which had had deep mesial caries at the first examination with weighted value of 3 and which it had been necessary to extract during the year was given a value of 4, i.e., an increased caries rating of 1.

Initial susceptibility to dental caries was determined by averaging two oral lactobacillus counts. Salivary samples were collected before brushing the teeth and before breakfast. All samples were obtained by chewing paraffin blocks and were diluted and plated within four hours on tomato-juice agar media pH 5.1 (Hadley's modification of Kulp's formula). After four days' incubation at 37.5° C., the oral lactobacilli were counted and an index of susceptibility was assigned to each boy on his chart.

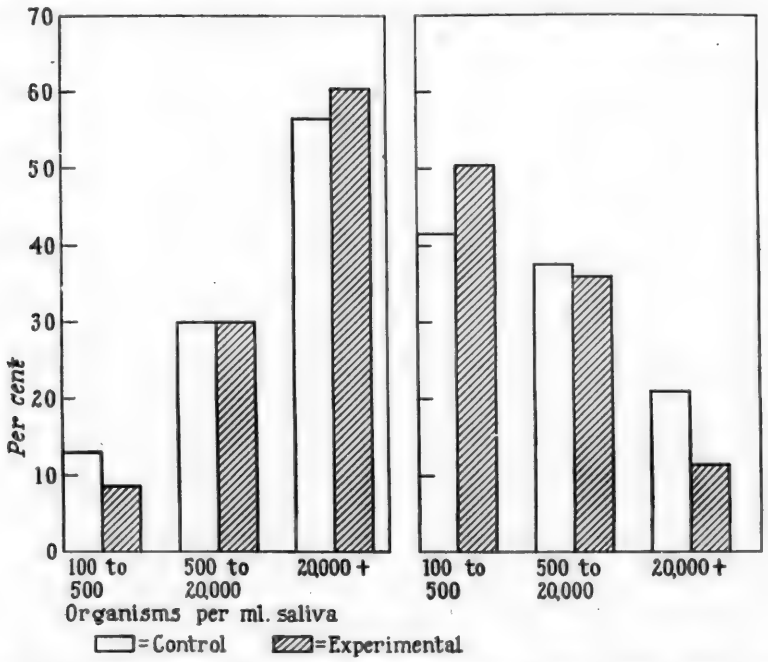

Fig. 1.—Graphic analysis of changes in oral lactobacillus counts before (left) and after (right) one year of use of penicillin-containing dentifrice. (All constantly negative counts eliminated.)

After the preliminary oral examinations and lactobacillus counts had been completed, all boys were supplied with individual four-ounce bottles of tooth powder and new toothbrushes. The occupants of five cottages were given tooth powder containing 1,000 units of penicillin per gram. Another group of three cottages, the occupants of which served as controls, used the same type of tooth powder without penicillin. They were instructed to brush their teeth twice each day. Other dentifrices used previously were removed

from the cottages, and instructions were given not to place a wet toothbrush in the bottle. Penicillin-containing tooth powder which became caked from moisture was replaced with new powder.

FINDINGS

An analysis of oral lactobacillus counts made for 163 boys revealed that thirty (18.4 per cent) remained constantly negative. Fig. 1 illustrates changes in oral lactobacillus counts at the end of one year in eighty boys of the experimental group and fifty-three boys of the control group. A general reduction in the occurrence of these organisms was observed. The decision to present these data as classified was purposeful in order that the degree of reduced

	Number of Boys	Initial Examination		Final Examination		Average Increase in Carious Surfaces
		Number of Carious Surfaces	Caries Weight	Number of Carious Surfaces	Caries Weight	
Experimental (Penicillin)	17	69	105	107	178	
	23	114	239	179	367	
	24	84	175	165	310	
	22	83	151	145	244	
	22	64	108	138	252	
Totals	108	414	778	734	1351	
Averages		3.83	1.88	6.80	1.84	2.97
Control	24	73	132	127	233	
	19	48	82	124	212	
	25	90	155	151	275	
Totals	68	211	369	402	720	
Averages		3.10	1.75	5.91	1.79	2.81

Fig. 2.—Summary data of caries experience in children during one year.

counts may be recognized. Note particularly that, as compared with the initial per cent found to belong to the 100 to 500 grouping, 50.2 per cent of the experimental and 41.5 per cent of the control group had become identified with reduced susceptibility to dental caries at the end of this study. Note also the marked reduction in the percentage of counts which were initially over 20,000 per milliliter of saliva.

Clinical evidence did not support the anticipated lessening of caries activity, if oral lactobacillus counts are to be considered as an index of the caries process. The compiled data of Fig. 2 are arranged according to cottages and represent the average number of cavities and extent of lesions in permanent teeth. No significant differences in the number of new carious surfaces nor progress in existent carious teeth can be ascertained from these data.

All boys were reared in vicinities without fluoride-containing waters.

No allergic manifestations to the use of penicillin in a dentifrice were observed.

From the amount of tooth powder which was used each month, it was observed that most of these boys did not follow the instructions given to them relative to brushing their teeth twice a day. The figures here represent the ordinary nonregimented methods of oral hygiene. The authors suspect that different results might be obtained with more regular use of penicillin tooth powder as evidenced by thirteen dental students who were selected because of their high caries susceptibility. These thirteen students had a marked reduction in acidophilus counts and only one carious lesion developed in a nine-month period.

The authors wish to thank the officials of Parmadale Children's Village of St. Vincent De Paul for their gracious cooperation, the Cleveland Dental Society for its financial support, Wyeth, Incorporated, for generous supplies of penicillin, the Comfort Manufacturing Company for generous supplies of tooth powder and the Buck X-Ograph Company for providing dental x-ray films.

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EFFECT OF SMALL AMOUNTS OF PENICILLIN ON THE ORAL BACTERIAL FLORA

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PENICILLIN inhibits the growth of a large percentage of the gram-positive group of organisms, and because the oral flora includes large numbers of these penicillin-sensitive organisms, we have attempted to determine the effects of relatively small dosages of penicillin administered orally on the oral bacterial flora. Long[1] has reported that penicillin given orally or ingested systemically greatly reduces, in a short time, the number of penicillin-sensitive organisms present in the oral cavity. As these organisms decrease, they are replaced by gram-negative organisms of the tribe Escherichieae, but Long[1] has shown that this group of organisms does not persist after treatment is discontinued. Webman, Hill, and Kniesner[2] noted, in studies of rats receiving penicillin in water, an increase in the occurrence of gram-negative organisms in conjunction with an increase in penicillin and sucrose dosages and a corresponding decrease in the incidence of oral lactobacilli.

In the following studies, a group of children from an institution were treated with small amounts of penicillin by the addition of 1000 units per gram to tooth powder, primarily in an attempt to reduce the occurrence of dental caries.[3] However, this paper is concerned with the *L. acidophilus* counts, determinations of number and presence of the predominating groups of aerobic organisms, and the occurrence of gram-negative bacilli of the tribe Escherichieae.

METHODS

Paraffin-stimulated saliva was collected in the morning before the teeth were cleaned and before breakfast. *L. acidophilus* counts were determined by the Hadley method. Specimens were diluted to obtain a countable number of organisms and were plated on blood agar. The resulting colonies were identified by routine bacteriological methods. The saliva specimen was also plated on Endo or eosin-methylene blue medium to show the presence of gram-negative organisms. These organisms were further identified by means of Voges-Proskauer, methyl red, and carbohydrate fermentation tests.

1. *L. acidophilus:*

The institutional children were divided into two groups. One group was given prepared tooth powder without the addition of penicillin, and the other received the same tooth powder with penicillin added. Both groups were un-

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aware of the contents of the tooth powder and were requested to use it once a day without supervision for a period of one year. An average of three *L. acidophilus* counts was taken before the experiment and after the end of the year. For purposes of comparison, all consistently negative *L. acidophilus* counts were eliminated from both groups. The control group, which did not receive any penicillin, was made up of fifty-three individuals and the experimental group of eighty.

Before the beginning of the experiment, we found the distribution of *L. acidophilus* to be fairly equal in both groups. After the use of the penicillin tooth powder for one year, the experimental group showed an increase in the number of children in a lower classification of *L. acidophilus* and a greater decrease of those previously in the high *L. acidophilus* counts than was shown by the control group (Fig. 1).

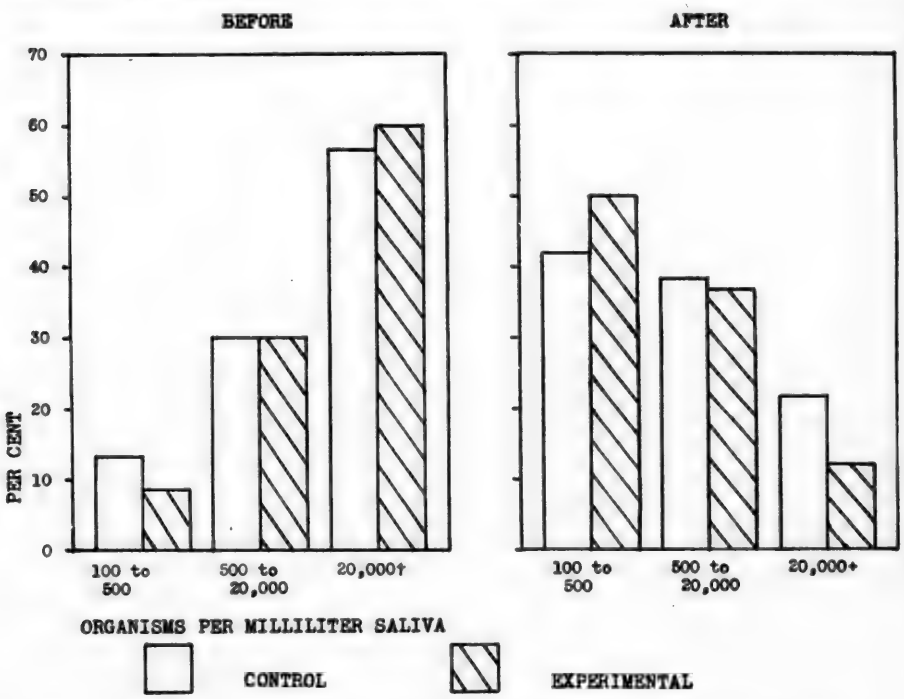

Fig. 1.—Comparison of *L. acidophilus* counts before and after use of penicillin dentifrice.

The decrease in numbers of *L. acidophilus* in the control group was more than would be expected, but the decrease in the experimental group was greater.

2. *Other organisms:*

Two groups were selected from the above persons, one group from each of the control and the experimental groups. These groups were further divided into groups of those having *L. acidophilus* counts of over 50,000 per ml. (++++) and those having negative *L. acidophilus* counts.

In the following charts, some organisms are presented as to their occurrence, whereas alpha and gamma streptococci, which were found to be present in all specimens, are presented as an average.

PERCENTAGE OCCURRENCE OF ORGANISMS IN SALIVA

	ACIDURIC STREPTOCOCCUS	BETA STREPTOCOCCUS	STAPHYLOCOCCUS	PNEUMOCOCCUS	MICROCOCCUS	DIPHTHEROIDS	NEISSERIA	A. AEROGENES	E. COLI	ACTINOMYCES	SUBTILIS	YEASTS	FUNGI
Control negative	96	35	48	61	83	44	87	17	0	52	22	13	4
Control ++++	90	60	40	20	80	50	100	0	10	60	30	10	60
Experimental negative	77	42	31	4	62	23	88	15	12	27		15	27
Experimental ++++	92	67	42	42	67	25	75	0	0	42	33	25	25
Total Groups													
Control	94	42	45	49	82	45	91	12	3	55	24	12	21
Experimental	82	50	34	16	63	24	84	11	8	32		18	26

AVERAGE COUNTS—ALPHA AND GAMMA STREPTOCOCCUS OCCURRING IN DILUTIONS OF 1:500,000

	ALPHA	GAMMA
Control negative	70	110
Control ++++	90	54
Experimental negative	49	60
Experimental ++++	51	74
Total Groups		
Control	78	79
Experimental	49	51

In the control groups, the occurrence of all other organisms is similar in high and low *L. acidophilus* counts. However, the *A. aerogenes* was associated only with the negative *L. acidophilus* counts and the gamma streptococcus showed less in the high *L. acidophilus* counts. In general, the number of other organisms was greater with the higher lactobacillus counts.

In the experimental groups, the organisms were definitely more numerous in the higher *L. acidophilus* grouping. Again the *A. aerogenes* occurred only in the negative group and *E. coli* was also found to occur more frequently in this group. The pneumococcus and aciduric streptococcus occurred less often in the same group.

Disregarding the *L. acidophilus* counts, and totaling the two groups as to those receiving penicillin treatment and those not receiving it, we found that the penicillin definitely reduced the number and occurrence of the penicillin-sensitive organisms of the mouth. It is of interest that these counts showed no correlation with the occurrence of yeasts.

3. *Production of gram-negative bacilli:*

Saliva specimens were checked at various times over a period of ten months for the presence of gram-negative bacilli of the tribe Escherichieae. These specimens were taken largely from the institutional group but were supplemented by

some taken from dental students. The specimens used were selected at random and while there were some taken from the same mouths at different times, the greatest number of counts were derived from different individuals.

	DATE	NUMBER OF COUNTS	OCCURRENCE OF GRAM-NEGATIVE BACILLI	PER CENT OF OCCURRENCE
Control group	April to September	101*	12	11
Experimental group	March to April	99	1	
	April to September	89*	27	
	December to January	102	0	9

*Groups supplemented by dental students.

There is very little difference in the per cent of occurrence of the gram-negative bacilli between those persons who were under treatment with the penicillin tooth powder and those who were not. However, the experimental group had a relatively high occurrence of gram-negative organisms because some of the supplementary dental students had received penicillin troches. The counts taken from December to April showed little or no occurrence of the gram-negative organisms while those taken from April to September, or the summer months, showed a comparatively high occurrence. This increase is correlated with a decrease in the *L. acidophilus* counts although there was also a decrease in other gram-positive organisms and a concomitant seasonal change.

The gram-negative organisms appeared only when the *L. acidophilus* counts were either low or negative, but did not necessarily occur with all low or negative counts. The organism which occurred most frequently (67 per cent of the gram-negative group) was *Aerobacter aerogenes.* Other organisms were classified roughly as species of Escherichia.

4. *Effect of increased dosages of penicillin:*

From a group of twenty-four dental students, eleven were selected as highly susceptible to caries by an average of three separate *L. acidophilus* counts. These eleven persons were given 500 units of penicillin per day in the form of troches for a period of one week only. At the same time they began to use penicillin tooth powder (1000 units per gram) and continued to use it throughout the experiment. *L. acidophilus* counts were made and the occurrence of gram-negative bacilli was noted at various times over a period of eight months.

	AVERAGE *L. acidophilus* COUNT PER ML. SALIVA	OCCURRENCE OF GRAM-NEGATIVE BACILLI (NUMBER OF INDIVIDUALS)
Before treatment	56,200	0
After treatment (troches)		
1 week	3,380	7
2 weeks	8,580	5
3 weeks	31,010	4
4 weeks	7,673	1
8 months	6,509	0

After treatment with the troches, there was a marked decrease in *L. acidophilus* which was not maintained. At the end of three weeks, the organisms were reasonably high but were gradually decreased by the continued use of the penicillin tooth powder.

The gram-negative organisms occurred in the same mouths on each count; however, the seven men, who first showed the presence of these organisms, lost them in varying periods of time. Those individuals who did not show any gram-negative organisms after treatment with the troches did not develop them at a later date. Again, the gram-negative group was found only with low or negative *L. acidophilus* counts, but not necessarily with all low or negative counts.

CONCLUSIONS

The number of oral *L. acidophilus* can be reduced by the proper and conscientious use of penicillin in tooth powder. The balance of the natural bacterial flora of the mouth is little changed by small amounts of penicillin, although the number and occurrence of organisms are reduced.

Gram-negative bacilli of the genera Aerobacter and Escherichia do increase in occurrence with a larger dose of penicillin but are not permanently maintained. Apparently, these organisms replace that part of the flora which is destroyed by the penicillin or by other factors involved in the normal changing of the flora. While the occurrence of gram-negative organisms was associated only with low or negative *L. acidophilus* counts, it appears indicative merely of a change in the balance of the normal flora.

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ANTAGONISTIC RELATIONSHIP BETWEEN ORAL ORGANISMS

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WITH the administration of large doses of penicillin, either orally or systemically, the oral bacterial flora changes to a predominance of gram-negative organisms of the tribe Escherichieae.[1] It was desirable to determine whether any of these gram-negative organisms are primarily antagonistic to the growth of *L. acidophilus*. *Aerobacter aerogenes* was selected from the gram-negative group for this study because it occurs more frequently in the mouth than the other organisms of the group and because Kesel, O'Donnell, Kirch, and Wach[2] have reported that filtrates of this organism will inhibit the growth of *L. acidophilus*.

METHOD

The tests were made by means of wells in agar plates. The wells were lined with celloidin membranes which were prepared by rinsing a flat-bottomed cylinder 2 mm. in diameter with a celloidin solution. When the celloidin had dried to a hard surface, the cylinders were immersed in cold water to facilitate the removal of the membrane. It was impossible to regulate the thickness of these membranes exactly, but, by the use of celloidin solutions of similar consistencies and by uniform technic, the membranes were fairly equal in thickness.

To show the presence of any antagonism between two organisms, one organism was seeded on the agar plate and the other in broth culture was placed inside the celloidin-lined well. Antagonism was demonstrated by the appearance of a zone of inhibition occurring around the well on the seeded plate. Tomato juice agar of pH 5 and a dextrose broth of the same pH were used in growing the cultures and in the tests. In some cases it was necessary to use blood agar and a corresponding broth of pH 7. Twenty-four-hour cultures of each organism were used for seeding the plates and filling the cups.

To determine whether the celloidin membrane would serve as a means of isolation of two different organisms and also permit the passage of any inhibitory materials, penicillin was placed in a cup and produced satisfactory inhibition of a susceptible organism. Broth alone inside the cup produced no areas of inhibition on seeded plates.

RESULTS

It was found by the method described that *L. acidophilus* inhibits *A. aerogenes* and that *A. aerogenes* organisms do not inhibit the growth of *L. acidophilus*. Twelve strains of *L. acidophilus* were tested, each inhibited *A.*

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aerogenes, but in varying degrees. One of the twelve strains on primary isolation from a susceptible person appeared very coccoid and was arranged in long chains. This strain produced the greatest inhibition of *A. aerogenes* and also inhibited, slightly, other strains of *L. acidophilus*. However, this same strain after repeated subculturing over a period of months lost its coccoid forms and its ability to inhibit other strains of *L. acidophilus*. *L. acidophilus* Type II (Hadley classification) produced the second greatest amount of inhibition of the *A. aerogenes* (Fig. 1).

Because these results appear to disagree with those of Kesel, O'Donnell, Kirch, and Wach,[2] it was decided to use the filtrates of eight-day cultures as he described in this method. It was found that a filtrate of *A. aerogenes* from a culture grown in a medium of pH 7 will inhibit the growth of *L. acidophilus*. The eighth-day culture and the resulting filtrate of *A. aerogenes* reached a final pH of 8 which is undoubtedly due to the ammonia produced, as Kesel, O'Donnell, Kirch, and Wach have described. However, filtrates of *L. acidophilus*

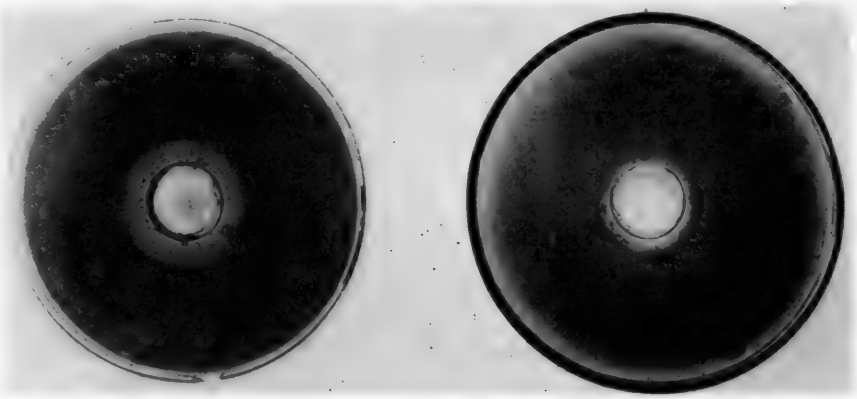

Fig. 1.—*A. aerogenes* is seeded on the outside agar. Cups at center contain *L. acidophilus* Type II on the left and Type I on the right. The clear area surrounding the cup indicates the zone of inhibition.

grown in media of optimum pH will in turn inhibit the growth of *A. aerogenes*, but filtrates taken from cultures of either organism grown at other than optimal pH will not produce inhibition. Inhibition was more pronounced when filtrates were used, if the filtrate was placed in the celloidin cup in the agar plate and refrigerated overnight before the plate was seeded with the organism to be tested. Possibly the agar absorbed more of the inhibiting substance and therefore produced a greater zone of inhibition.

Several organisms have been tested for possible antagonism. The only organism found that would inhibit *L. acidophilus* was *Pseudomonas aeruginosa*. This is maybe due to the production of pyocyanase, an antibacterial substance, which is slightly inhibitory to some gram-positive organisms. Besides inhibiting *A. aerogenes*, *L. acidophilus* will inhibit *Escherichia coli* and to a lesser degree, some strains of Sarcina. Extent of inhibition is noted in Table I.

TABLE I
INHIBITION OF L. ACIDOPHILUS

SEEDED ON AGAR	INHIBITION PRODUCED BY	GROWTH	INHIBITION
L. acidophilus Type II	Penicillin 10 units		4.5 cm.
L. acidophilus Type II	*A. aerogenes*	–	–
L. acidophilus Type II	*A. aerogenes* filtrate pH 8		4.0 cm.
L. acidophilus Type II	*L. acidophilus* # 20	+	3.1 cm.
L. acidophilus Type II	*Ps. aeruginosa*	+	2.5 cm.

To date we have found no organisms that *A. aerogenes* cultures will inhibit.* However, there are several organisms which will inhibit *A. aerogenes*. In this group, successively, are the *L. acidophilus*, then hemolytic *Staphylococcus aureus* and *albus*, *Proteus vulgaris* and some strains of Sarcina (Table II).

TABLE II
INHIBITION OF A. AEROGENES

SEEDED ON AGAR	INHIBITION PRODUCED BY	GROWTH	INHIBITION
A. aerogenes	*L. acidophilus* # 20	+	5.5 cm.
A. aerogenes	*L. acidophilus* Type II	+	3.7 cm.
A. aerogenes	*L. acidophilus* 4646	+	2.3 cm.
A. aerogenes	*L. acidophilus* Type I	+	2.5 cm.
A. aerogenes	*L. acidophilus* filtrate pH 5		3.1 cm.
A. aerogenes	*Staph. aureus*	+	4.2 cm.
A. aerogenes	*Staph. albus*	+	4.7 cm.
A. aerogenes	*P. vulgaris*	+	2.8 cm.
A. aerogenes	Sarcina	+	2.4 cm.

Other organisms, commonly found in saliva, were tested for reciprocal antagonistic influence. Streptococcus, neisseria, pneumococcus, yeast, and subtilis produced no effect with either *L. acidophilus* or *A. aerogenes*.

P. vulgaris was selected to test the possibility of proteolytic activity influencing inhibition. It was found that it had no effect on the *L. acidophilus*, but it did inhibit the growth of *A. aerogenes*.

All organisms, with the exception of *A. aerogenes*, placed within the celloidin cup continued to remain viable while surrounded by another growing organism. *A. aerogenes* did not survive when surrounded by *L. acidophilus* on tomato juice agar pH 5. After eighteen to twenty hours of incubation, the pH of both the agar and the broth culture inside the cup were reduced to 4.1. *A. aerogenes* will not grow at this acidity.

*Since the original writing of this paper, it has been found that agar plugs surrounded by poured agar can replace the celloidin-lined wells. Numerous other organisms have been tested for their antagonistic relationship to *L. acidophilus* and *A. aerogenes*.

No other growing organisms were found that would inhibit *L. acidophilus*. However, *L. acidophilus* will inhibit, in addition to those organisms previously mentioned, *Salmonella typhosa*, *Gaffyka tetragena*, *Klebsiella pneumoniae*, *Staph. citreus*, some strains of micrococcus, *Sarcina lutea*, *Serratia marcescens*, and *Lactobacillus casei*.

One organism was found to be inhibited by *A. aerogenes*. This was *Sarcina lutea*.

The growth of *A. aerogenes* was inhibited also by *L. casei* and very slightly by some strains of micrococci.

It appears that *L. acidophilus* shows the greatest inhibitory influence on gram-negative organisms of the family Enterobacteriaceae and on the family Micrococcaceae.

The pH was measured in the broth culture in the cup, in the zone of inhibition, and in the surrounding media, after twenty-four-hour incubation. In all cases, the pH of the inhibition zone was between that of the broth and that of the surrounding agar. When *A. aerogenes* was seeded on the outside agar, the pH of the agar was raised, and when *L. acidophilus* was placed on the outside, the pH of the agar was lowered.

To test the possibility that the inhibition was caused by the acid carried into the cup in the twenty-four-hour broth cultures, fresh broth was placed in the cup and inoculated with organisms from agar slants. The results were the same.

It was suggested that the inhibition of *A. aerogenes* might be due to lactic acid produced by *L. acidophilus* as a result of carbohydrate degradation. The normality of the acid produced in the cup by organisms that inhibited *A. aerogenes* was never more than 0.06 N. When a 0.4 N solution of reagent grade lactic acid adjusted to a pH of 4.6 with sodium hydroxide was placed in cups on seeded plates, both *A. aerogenes* and *L. acidophilus* were inhibited. Less concentrated solutions of lactic acid produced no inhibition. It would therefore seem that *L. acidophilus* in the cup did not form sufficient lactic acid to cause the inhibition of *A. aerogenes.*

CONCLUSION

There is an antagonism between some of the organisms commonly found in saliva:

L. acidophilus inhibited the growth of *A. aerogenes* and viable *A. aerogenes* did not inhibit the growth of *L. acidophilus.*

Filtrates of some cultures of *A. aerogenes* will inhibit the growth of *L. acidophilus* and filtrates of some cultures of *L. acidophilus* will inhibit the growth of *A. aerogenes.*

The degree of the antagonistic reaction between organisms is influenced by the pH of the medium in which the organisms are grown.

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OBSERVATIONS ON LACTOBACILLUS COUNTS AND DENTAL CARIES IN MENTAL PATIENTS DURING INSULIN COMA THERAPY

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SINCE it is a common practice in mental institutions to administer large quantities of sugar in terminating induced therapeutic insulin coma, it appeared important to determine whether the dental health of patients subjected to this treatment would be affected adversely. In the present investigation, a direct approach to this problem has been made through a study of changes in clinical dental caries and in the salivary lactobacillus counts of a group of individuals undergoing insulin hypoglycemia therapy for various mental disorders.

While it cannot be concluded that *Lactobacillus acidophilus* is the primary etiological agent in dental caries,[9] the lactobacillus count has been generally accepted as an indicator of susceptibility to the disease. A relationship has been found between the number of lactobacilli in the saliva and the activity of the dental caries process.[1-5, 7, 8, 11] The correlation of high lactobacillus counts with caries susceptibility and of negative counts with resistance to caries has been stated to be 90 per cent positive.[3]

Experimental studies have demonstrated a direct relationship between proliferation of oral lactobacilli, increase in caries activity, and sugar consumption.[5, 8] Hill[6] believes that this interrelationship is shown by the great majority of people.

PROCEDURE

Patients admitted to the hypoglycemia ward of the Cleveland State Hospital were observed during the entire course of their therapy. The capacity of the ward was 10 beds, and the period of treatment varied from two to thirteen weeks, depending upon the progress of the subject, so that it was possible to collect data for only 25 cases in seven months of observation. In spite of attempts to exclude patients who would not submit to regular observation, there are gaps in the available data which result from temporary indisposition on the part of many of the subjects.

Salivary analyses.—At least two consecutive daily specimens of saliva were obtained for bacterial analysis before treatment, in order to establish the initial level of *L. acidophilus*. Analyses were performed once weekly thereafter. The samples were obtained on the morning following a day on which no treatment had been given so that there was no unusual consumption of sugar for at least 36 hours preceding the collection. The bacterial counts were estimated by a modification of the method originally described by Hadley.[5] All specimens of saliva were collected in the early morning before any food or drink was taken and before the teeth were brushed. The subject was allowed to chew a small piece of paraffin wax until it became soft. All of the saliva stimulated by con-

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tinued chewing during the following three minutes was discharged into a sterile bottle. The bottle was closed tightly and was kept in a refrigerator for 3 or 4 hours before delivery to the laboratory.

A sterile technic was followed during the analysis. The saliva was shaken vigorously in the bottle on a mechanical device which produced a relatively homogeneous suspension in a few seconds. One-tenth milliliter of a 1:10 saline dilution of the saliva was placed on tomato-juice agar media and was spread over the surface of the plate with a glass crook. After incubation at 40° C. for 3 days, colonies were identified grossly or microscopically and were counted. The count was expressed as the number of organisms per milliliter of whole saliva.

Clinical examinations.—Before therapy was instituted, each patient was examined with the aid of a dental mirror and exploring tine. Bite-wing radiograms were taken if the subject was cooperative. The number of D.M.F. (decayed, missing, or filled) tooth surfaces was recorded. At the conclusion of treatment the patient was re-examined by the same investigator, and changes were noted and compared.

Insulin.—The treatment was given on five mornings each week. The subjects received an injection of commercial insulin at 7 A.M., and by 9 A.M. they were usually in some stage of hypoglycemic coma. After 30 to 60 minutes in this condition, the hypoglycemia was relieved by the administration of some form of sugar preparation. The dose of insulin was small at the outset but it was administered in increasing amounts at each treatment, the amount of the dose depending on the resistance of the patient to the induction of coma.

Sugar solutions.—The sugar used in greatest preponderance was sucrose. A solution was prepared volumetrically by adding 500 milliliters of granulated cane sugar to an equal volume of tap water. The mixture was stirred and boiled for a few minutes to hasten dissolution and the resulting syrup was cooled to tolerable temperature before use. The degree of hydrolysis of the sucrose to its monosaccharide components is negligible with this procedure.

To terminate the hypoglycemia, the sucrose solution was administered either from a cup or by gavage tube. In cases where the patient was unable to cooperate for oral or tube administration, a small dose of sterile glucose solution was injected intravenously. When the patient had become more rational the sucrose was given by one of the above mentioned means. Each subject received sucrose solution every morning, followed by additional quantities later in the day if a relapse into hypoglycemia occurred.

Diet.—The diet supplied to these patients was high in carbohydrate content, and the portions of all foods were unusually large. A voracious appetite commonly develops in individuals undergoing insulin therapy.

Toothbrushing.—No recommendations were made regarding toothbrushing or care of the mouth.

Records.—Records were kept of the daily dosage of insulin, the intake of sugar as well as the mode of its administration, lactobacillus counts, changes in weight, and changes in the oral cavity.

OBSERVATIONS AND FINDINGS

Salivary analyses.—On the basis of their initial lactobacillus counts the subjects could be divided into two groups, which were designated A and B. Group A comprised 6 individuals whose saliva contained from 0 to 10,000 lactobacilli per milliliter; Group B comprised 19 individuals whose counts were over 10,000. The counts for all cases ranged from 0 to more than 1,500,000.

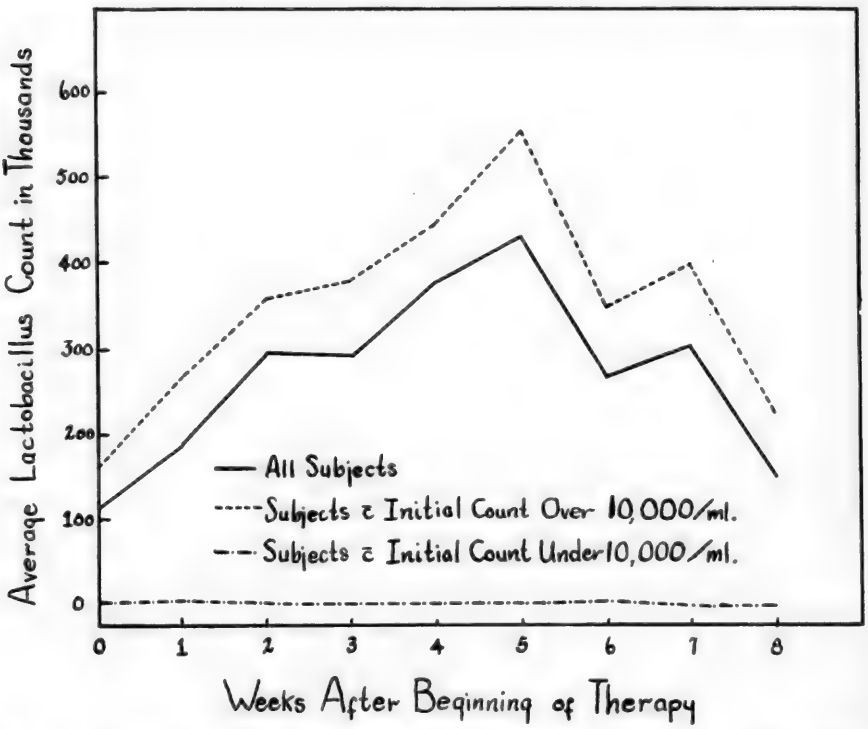

Fig. 1.—Average lactobacillus count in thousands by weeks after beginning of insulin therapy.

The mean counts calculated for each week of active therapy are presented in graphic form in Fig. 1. The gross picture for all cases seemed to indicate that the mean count rose progressively from week to week, reached a peak after five weeks, and then apparently declined. When the data were considered by groups it became evident that this rise could be attributed to the patients in Group B, who, on the basis of their counts, would be expected to show greater susceptibility to caries. The subjects in Group A did not exhibit a rise in count sufficient to change their classification, and members of this group were considered to be relatively resistant to caries.

The data for the susceptible group were tested for statistical significance, and a negative result was obtained. If the oral lactobacilli were increased by any feature of the therapy, it would be expected that the number of rising counts would exceed the number of declining counts. The number and percentage of

cases in which the count increased over the initial base line, and also over the preceding weekly count, appear in Table I. Analysis showed that these values could have been obtained by random sampling. In consideration of the small number of cases under study, the attachment of significance to the increase manifested in Fig. 1 is not justified on a statistical basis.

TABLE I

NUMBER AND PERCENTAGE OF CASES WHOSE LACTOBACILLUS COUNT ROSE CONSECUTIVELY DURING THERAPY

WEEKS	1	2	3	4	5	6	7	8
Referred to initial count	9/16	11/15	7/13	9/15	5/11	4/10	3/9	4/6
Per cent rising	56	73	54	60	45	40	60	33
Referred to previous count	9/16	8/13	4/11	7/12	5/9	4/8	3/7	3/7
Per cent rising	56	62	36	58	56	50	43	43

The denominator of each fractional expression represents the number of cases for whom counts were obtained on consecutive weeks; the numerator represents the number of counts in each group that were higher than their respective level of reference.

Clinical caries.—Final examinations could be performed on only 11 subjects. The net increases in number of D.M.F. surfaces are given in Table II. The number of subjects was too small to permit the drawing of a definite conclusion, and no statistical difference between the two groups could be demonstrated. While it was not possible to secure adequate comparable controls, it is the opinion of the authors that the net D.M.F. values are considerably higher than those which might be expected in normal populations over similar short periods of time.

TABLE II

NET INCREASES IN D.M.F. SURFACES AFTER CONCLUSION OF INSULIN COMA THERAPY

CASE	GROUP A (LOW-COUNT)	GROUP B (HIGH-COUNT)
1		0
3	19	
5		2
13		22
15	6	
16		11
17		0
18	6	
19		9
23		18
25		28
Total	31	90
Mean per person	10.3	11.25

Sugar consumption.—The weekly intake of sugar solutions ranged from 2,500 to 5,000 milliliters in different patients. This is equivalent to 3 to 6.5 pounds of sugar per week, in addition to that normally present in the high carbohydrate diet which these patients received. The maximum total amount of sugar consumed here is probably not exceeded by the most avid consumers of candy in normal populations.

No subject received sugar by only one of the pathways described, and no correlation could be found between the individual lactobacillus counts, clinical caries, and the mode of sugar administration.

Insulin.—The quantity of insulin administered to each patient was increased until a level was reached, usually within the first week, at which the patient went into coma. The recorded doses were added by weeks and the mean weekly dose was calculated for each patient. The value of the mean weekly dose ranged from 521 units to 7,043 units. The average mean weekly dose for all 25 cases was 2,075, with a standard deviation of 1,325.

The average mean weekly dose for Group A was 1,410 with a standard deviation of 340, and for Group B it was 2,285 units with a standard deviation of 346. The difference between these values was tested for significance. The critical ratio was 2.36. The probability that this difference would be reached or exceeded by chance was found to be 0.02, or 1 in 50. The findings were considered significant.

The numbers of cases in each group, whose mean weekly insulin dose was above or below the grand mean for all cases, are presented in Table III. This result was evaluated by the Chi-square test, and the probability that the observed values were independent of each other was found to be 0.02. This further confirmed the significance of the findings.

TABLE III
DISTRIBUTION OF CASES BY GROUPS WITH RESPECT TO THE AVERAGE MEAN WEEKLY INSULIN DOSE FOR ALL CASES

	GROUP A (LOW-COUNT)	GROUP B (HIGH-COUNT)
Number above the mean	0	10
Number below the mean	6	9

It may be stated, therefore, that in this study a definite correlation has been found between a low initial lactobacillus count and the relative sensitivity of the patient to induced insulin coma.

DISCUSSION

While the technical variation in lactobacillus counts throws some question upon the reliability of this method as a quantitative test for caries susceptibility,[10] it is of interest to note that no increase in count was manifested by patients in Group A during their exposure to the sucrose solution, even though lactobacilli frequently were present in numbers sufficient to serve as adequate in vivo inocula. Since the substrate was supplied generously to all mouths, it appears that some unknown, and perhaps transient, resistant factor may have been operating in Group A to prevent proliferation of the organisms. It should also be noted that Case 3 (Table II), whose count was below 500 during treatment, had a count of 200,000 when examined two months after treatment had been discontinued. It was not possible to determine when the new carious lesions developed.

The correlation between lactobacillus counts and insulin dosage was unforeseen. There was no quantitative relationship between the magnitude of the counts and the actual dosage of insulin. No explanation is offered regarding this phenomenon. Further study is indicated to determine whether the low counts are associated with a sensitivity to insulin per se or with a resistance to

coma when the blood sugar has been lowered by the insulin, and also to ascertain whether the count may be used to predict the approximate dose of insulin that will produce coma in the subject. The necessity of obtaining larger numbers of experimental and control subjects for proper evaluation of future observations has been recognized.

SUMMARY

1. Salivary analyses of mental patients before hypoglycemia therapy indicated that they could be divided into two distinct classes on the basis of their lactobacillus counts. Those with less than 10,000 lactobacilli per milliliter were designated as "low-count," while those with 10,000 or more lactobacilli per milliliter were designated as "high-count."

2. An apparent rise which was observed in the weekly lactobacillus counts of the high-count group was not statistically significant. However, no rise in count was observed in the low-count group.

3. The incidence of new clinical caries during the period of treatment was not significantly greater nor more extensive in either group.

4. No significant correlation could be demonstrated between the magnitude of the sugar intake and the development of new caries in either group.

5. The dosage of insulin required to produce coma was consistently lower in the low-count group than the grand mean dosage for all cases. The insulin dosages for the high-count group varied at random with respect to the grand mean. All cases whose insulin dosage was greater than the mean were members of the high-count group. These findings were significant by statistical tests.

The authors are grateful to Dr. Maurice B. Gordon, Clinical Director, and Dr. Anthony H. Wichert, Chief of Dental Department of the Cleveland State Hospital, for their cooperation, and particularly to Dr. Thomas J. Hill of the Institute of Pathology, who made many important suggestions throughout the course of the investigation.

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OBSERVATIONS ON EXPERIMENTAL DENTAL CARIES

III. The Effect of Dietary Lactic Acid

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THE decalcification of enamel by acids of bacterial origin, particularly lactic acid,[1] is still thought by many investigators to be the original lesion in dental caries. McClure[2] has observed a pronounced destructive effect of dilute solutions of hydrochloric and lactic acids on the enamel and dentin of rat molars. Gortner, Restarski, Bieri, and McCay[3] have also shown that drinking solutions of lactic and other acids produces marked decalcification of the rat and hamster molars.

On the other hand, Gortner, McCay, Restarski, and Schlack[4] have observed that oxalic acid solutions, unlike solutions of other acids, do not produce etching of the rat enamel. On the contrary, oxalic acid or its sodium salt diminishes or prevents the enamel decalcification that might be expected by the simultaneous ingestion of phosphoric acid and citric acid solutions. This protective role of oxalic acid against enamel decalcification was found to be due to the formation of calculuslike deposits, presumably of calcium oxalate, on the molars. Taking into consideration these and some other observations, Schlack, Howell, Taylor, Berzinskas, and Aborn[5,6] have investigated the effect of oxalates, administered in the food and in the drinking water, on caries activity in the albino and cotton rats. These workers found no protective action of the oxalates against the incidence or extent of carious lesions.

We are reporting here the results of an experiment on the effect of dietary lactic acid, incorporated in the food, and in the drinking water, on caries activity in the hamster.

EXPERIMENTAL

Forty-five Syrian hamsters, between 21 and 25 days of age, from litters of a colony maintained on a diet of Purina Laboratory Chow* and raw milk, were distributed into three groups of fifteen animals each (eight males and seven females). The animals were set in screen bottom cages without bedding, and reared for 100 days on the following rations:

Group 1 (Diet 1): ground yellow corn† 40 per cent, sucrose 20 per cent, cornstarch 5 per cent, potato starch 5 per cent, powdered whole milk 19 per cent, ether-extracted yeast 5 per cent, alfalfa meal 5 per cent, and sodium chloride 1 per cent.

Group 2 (Diet 2): to each 100 gm. of Diet 1 were added, and thoroughly mixed, 0.057 c.c. of lactic acid (80 per cent). Groups 1 and 2 were given distilled water to drink.

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*From Ralston Purina Co., St. Louis, Mo., U.S.A.

†The particle size of the ground yellow corn was distributed as follows: 100 per cent through a 12.5-mesh screen; 63 per cent through a 25-mesh screen, and 31 per cent through a 50-mesh screen.

Group 3 (Diet 3): this group was fed Diet 1, but here each 100 c.c. of the distilled water given to drink contained 0.050 c.c. of lactic acid (80 per cent). The diets as well as the drinking fluids were all available ad libitum, and the animals were weighed every week. These experiments were carried out between the months of November and February.

The amounts of lactic acid given in the diet to Group 2 and that supplied in the water to Group 3 were calculated in such a way that the animals of these two groups would ingest daily the same amount of lactic acid. This calculation was made after observation of the average daily amount of food and water consumed by the hamster. The pH of the diets mixed with distilled water was determined with a glass-electrode potentiometer. The diets and the fluids had the following pH: Diet 1, 5.55; Diet 2, 5.12; distilled water, 6.8; distilled water + lactic acid, 3.1. Throughout the whole experimental period the lactic acid was added to the food (Diet 2) and to the water (Diet 3) every day.

On completion of the experimental period the animals were sacrificed and autopsy was performed on them. After fixation in 10 per cent formalin, the jaws were prepared for examination in the usual way. The degree of enamel decalcification was evaluated in accord with the method devised by Restarski, Gortner, and McCay,[7] and the carious lesions were recorded and scored using the chart described by Keyes.[8] Ground sections were prepared from several molars of the different groups.

RESULTS

The animals from the three groups exhibited the same healthy appearance. Likewise, the growth rate of the three groups was essentially the same, as it can be seen in Fig. 1. Møllgaard, Lorenzen, Hansen, and Christensen[9, 10] have reported that lactic acid, either incorporated in the diet or formed in the intestines in increased amounts by ingestion of skim milk acidified with lactobacillus, produces in the pig a marked increase in the absorption of calcium and phosphorus, and considerably promotes the growth and health of this animal. However, under the conditions of our experiment we found that, in the hamster dietary, lactic acid does not improve the growth or health of the animal. This difference may be due to the fact that all or most of the lactic acid supplied to the hamsters was constantly neutralized or combined in such a way that it could not reach the intestines in the free state, and there exert any apparent beneficial action on the growth. Otherwise, some physiological and biochemical differences with respect to lactic acid that might exist between the two animal orders could account for this discrepancy.

The autopsy revealed no gross changes other than those found in the oral cavity. Various degrees of alveolar resorption were found in several animals, but there was not significant difference in the incidence or extent of resorption among the three groups.

In Group 1 the common yellow-brown stain of the enamel cuticle, unrelated to caries activity, was found on all surfaces of the molars. In this group there was no evidence of enamel decalcification in any of the molar surfaces (grade 0).

In Group 2 the stain was present mainly on the lingual surfaces of both upper and lower molars. Some buccal and many occlusal surfaces appeared stainless and slightly chalky; this was especially true in the occlusal fossae. This indicates that the lactic acid incorporated in the food acted, at least slightly, on the enamel of those surfaces which are most favorable for food retention. In accord with Restarski, Gortner, and McCay's method[7] this group was also grade 0. In Group 3 the stain was almost completely absent. The enamel of most surfaces appeared stainless, somewhat chalky, and sometimes slightly etched. The cusps of the molars appeared somewhat shorter, thus giving a shallower appearance to the fossae. In this group, furthermore, lines of fracture and brittleness of

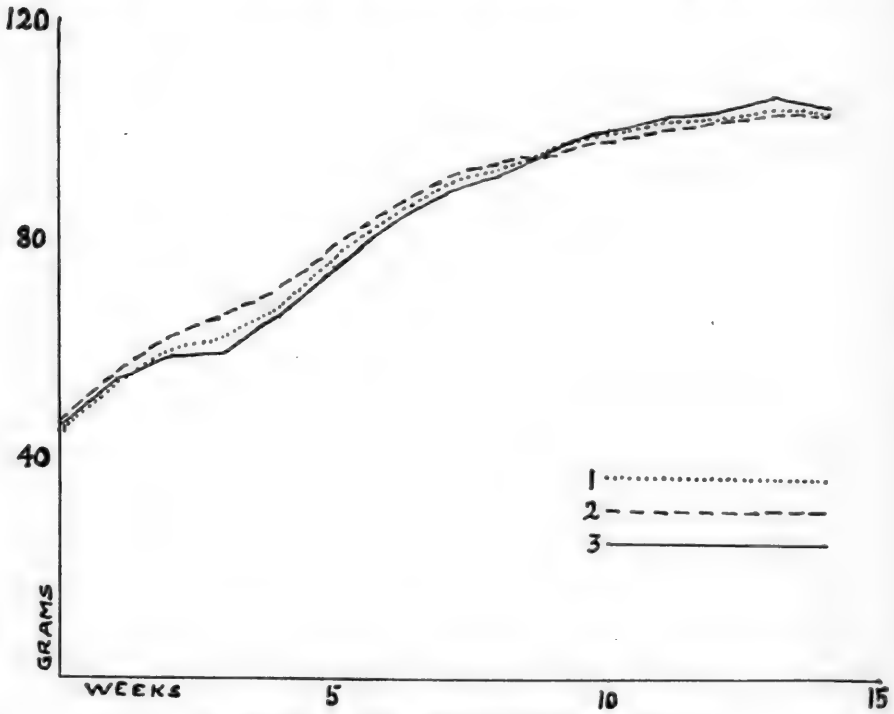

Fig. 1.—Average growth curves of the three groups.

the enamel were frequently observed. It is apparent that all these changes, found in both jaws with about the same intensity, were due to the lactic acid contained in the water given to Group 3. The enamel decalcification of the lingual surfaces in this group was grade 2.

As it can be seen from Table I, there was no significant difference at all in the incidence or extent of carious lesions among the three groups. Comparing the caries activity in the two jaws (Table II), Group 1 exhibited slightly higher incidence and extent of lesions in the maxilla. In Group 2 the caries activity also was significantly higher in the maxilla. In Group 3 the incidence of lesions

was only slightly higher in the maxilla, but the extent of the lesions was significantly higher than in the mandible. Comparing the incidence of occlusal, buccal, and lingual carious lesions in the various groups, Table III shows that the lingual surfaces were the least affected in all the three groups, and that there was a significant higher incidence of lesions in the occlusal than in the buccal surfaces, this being especially so in Group 2.

TABLE I
CARIES INCIDENCE IN THE THREE GROUPS

	GROUP 1			GROUP 2			GROUP 3		
	♂♂	♀♀	♂♂ + ♀♀	♂♂	♀♀	♂♂ + ♀♀	♂♂	♀♀	♂♂ + ♀♀
Number of experimental animals	8	7	15	8	7	15	7*	7	14
Number of animals affected	8	7	15	8	7	15	7	7	14
Number of carious molars	62	62	124	62	51	113	57	60	117
Average number of carious molars	7.7	8.8	8.2	7.7	7.3	7.5	8.1	8.6	8.3
Number of carious lesions	90	90	180	98	73	171	90	89	179
Average number of carious lesions	11.2	12.8	12	12.3	10.4	11.4	12.9	12.7	12.8
Average scores of carious lesions	7.0	6.8	6.9	8.7	5.9	7.3	7.9	7.9	7.9

*One of the eight original males of this group died at the beginning of the experiment.

Table I also shows that although in Group 2 there was a higher incidence and extent of caries in the males than in the females, this was not the case in Groups 1 and 3. Thus, in these experiments, no sex difference was consistently found in relation to caries activity. Through the microscopic study of ground sections, signs of acid action (transverse striations of the prisms, cloudy enamel) were found, combined or not with caries, at the very bottom of many occlusal fossae in Groups 2 and 3.

TABLE II
CARIES INCIDENCE IN THE TWO JAWS

	GROUP 1 15 ANIMALS		GROUP 2 15 ANIMALS		GROUP 3 14 ANIMALS	
	MAXIL.	MANDIB.	MAXIL.	MANDIB.	MAXIL.	MANDIB.
Number of carious molars	67	57	70	43	66	51
Average number of carious molars	4.5	3.8	4.7	2.9	4.7	3.6
Number of carious lesions	93	87	106	65	96	83
Average number of carious lesions	6.2	5.8	7	4.3	6.9	5.9
Average scores of carious lesions	7.3	6.8	10.4	4.5	8.5	4.8

TABLE III
PERCENTAGE OF OCCLUSAL, BUCCAL, AND LINGUAL CARIOUS LESIONS IN THE THREE GROUPS

LESIONS	GROUP 1	GROUP 2	GROUP 3
Occlusal	76.2	88.3	76.0
Buccal	22.6	9.4	22.9
Lingual	1.2	2.3	1.1

DISCUSSION

The incidence of caries in the various surfaces in the three groups may be compared and correlated with the signs of enamel decalcification present in certain surfaces in Groups 2 and 3. Groups 1 (water) and 3 (lactic acid in the water) exhibited essentially the same incidence of caries in the various surfaces. Thus the lactic acid solution given to Gorup 3 was able to produce an enamel decalcification which did not alter the susceptibility of the decalcified surfaces to dental caries. On the other hand, in Group 2 (lactic acid in the food), the occlusal surfaces, being less affected by acid than in Group 3 but more than in Group 1, exhibited higher caries incidence than in Groups 1 and 3, at expenses of much lower incidence in the buccal surfaces (Table III). This difference, the origin of which is not clear, is, however, within nearly the same total incidence and extent of caries present in all the three groups.

Thus, under the conditions of our experiment, dietary lactic acid produced enamel decalcification but failed to alter, in any direction, the incidence or extent of carious lesions. On the other hand, if the incidence of caries were to be at least partially increased by the presence of lactic acid in the bottom of the occlusal fossae, one could say that Group 2 exhibited a higher incidence of occlusal lesions than the other groups; for in Group 2 the lactic acid mixed in the food was forced rapidly during the process of mastication into the occlusal fossae, without the saliva having had time to neutralize it. Perhaps this might explain the higher incidence of occlusal cavities in Group 2, but not the decrease of buccal lesions in this group (Table III) to a level far below the percentage of buccal lesions in Groups 1 and 3.

The fact that we found gross and microscopic evidence of acid action in many occlusal fossae, including the bottom of them, and combined or not with caries, in Groups 2 and 3, leaves no doubt that the dietary lactic acid in many instances reached the bottom of the fossae. In spite of this, we have seen that there was no significant difference in the incidence or extent of lesions among the three groups. This fact shows that, in these experiments, the dietary lactic acid, which could reach even the bottom of the fossae, did not play any important role in the development or progress of dental caries.

SUMMARY

The effect of dietary lactic acid on dental caries was studied in three groups of newly weaned hamsters, which were reared for 100 days on the experimental diets. Group 1 (control) was fed the cariogenic ration, and distilled water. Group 2 received the cariogenic diet to which had been added 0.057 c.c. of lactic acid (80 per cent) per 100 gm. of food, and distilled water. Group 3 was fed the cariogenic diet, and distilled water to which had been added 0.050 c.c. of lactic acid (80 per cent) per 100 c.c. of water.

No significant differences were found in the incidence or extent of carious lesions among the three groups. The animals from all the groups exhibited essentially the same health appearance and the same growth rate.

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IN VITRO STUDIES OF THE DENTAL CARIES INHIBITING PROPERTIES OF SOME SELECTED NITROFURAN COMPOUNDS

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WHILE the precise etiology of dental caries is still a matter of conjecture, there is widespread agreement that dental decay is essentially a posteruptive disease which affects the calcified tissues of the teeth. The mechanism appears to be, in part at least, a dissolution of the inorganic salts of the tooth associated with a decomposition of the organic matrix. The lesions develop predominantly in the regions of the pits and fissures, gingival third, and near the contact points of the teeth. Irrespective of the anatomical location, however, the caries process begins at the external surface of the tooth and extends into the structure.[1] The dissolution of the tooth structure is believed to result from the acids produced during the fermentation of ingested foodstuffs by certain acidogenic microorganisms present in the mouth. The dental enamel as well as the dentin and cementum is composed principally of complex calcium salts which by their very nature are insoluble in saliva but soluble in acids. Since the enamel-producing ameloblastic layer of cells disappears following the eruption and articulation of a tooth, enamel once dissolved cannot be regenerated. Prevention of the dissolution of enamel has become, therefore, a prime requisite in the control of dental caries.

The demonstration by Dodd and Stillman,[2] that certain furan derivatives exerted a bacteriocidal and bacteriostatic action in vitro against a number of gram-negative and gram-positive pathogenic bacteria when a nitro group was added to position 5 in the furan ring (Fig. 1), suggested to the authors that the nitrofurans may have value in the prevention and control of dental caries. The clinical findings of Snyder, Kiehn, and Christopherson[3] and Downing, Hanson and Lamb[4] among others showed that one of the nitrofuran compounds was effective as an antibacterial agent in vivo. In these and other reported in vivo studies, Furacin (5-nitro-2-furaldehyde semicarbazone) (Fig. 1) has been the compound of choice because of its stability, low toxicity, and nonirritating qualities. In addition, its antibacterial activity is not reduced in the presence of organic matter, and it has been ingested by man in quantities of 300 milligrams per day without any discernible toxic effects.[5, 6]

Preliminary investigations performed in our laboratory revealed that the addition of 500 micrograms of 5-nitro-2-furaldehyde semicarbazone to 10 c.c. aliquots of human saliva completely inhibited acid production in vitro in 64 per

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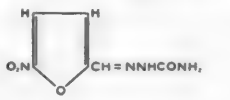
cent of the cases in which caries activity was present in the control aliquot, as evidenced by the Fosdick, Hansen, and Epple test.[7] The addition of either 1 mg. or 2 mg. quantities of this compound completely prevented acid production in 94 per cent and 100 per cent, respectively, of the samples which were caries active.

The present in vitro study was undertaken to compare the inhibitory effects of Furacin and nine related nitrofuran compounds* on acid production in the saliva and on the growth and acid production of a pure culture of a strain of the *Lactobacillus acidophilus* recovered from a carious lesion. The nine compounds used, in addition to Furacin, were 5-nitro-2-furaldoxime, 5-nitro-2-furyl n-amyl ketone, 5-nitro-2-furaldehyde-2-B-methyl semicarbazone, 5-nitro-2-furaldehyde acetyl hydrazone, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, 5-nitro-2-furaldehyde-(5′-B-hydroxethyl)-semioxamazone, 5-nitro-2-furfurylidene acetone semicarbazone, 5-nitro-2-furaldehyde acethydrazone trimethyl ammonium chloride, and the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride. Each of them differed from Furacin per se in the composition of the side chain on the furan ring, as can be seen in Fig. 2.



5-NITRO-2-FURALDEHYDE SEMICARBAZONE
(FURACIN)

Fig. 1.—Structural formula of Furacin.

MATERIALS AND METHODS

The Fosdick, Hansen, and Epple[7] test for dental caries activity was chosen to study the effect of the ten selected nitrofuran compounds on acid production in the saliva. In this test the degree of dental caries activity is determined by analyzing two 10 c.c. aliquots of a saliva sample for soluble calcium. One aliquot is tested immediately following the collection of the sample, while the second is added to a sterile brown vial containing dextrose and tricalcium phosphate. The vials are sealed and agitated for four hours in a shaker apparatus submerged in a water bath which is maintained at 37° Centigrade. The difference in the amounts of soluble calcium recovered from the incubated and unincubated aliquots offers a measure of the degree of dental caries activity.

Sixty samples of 50 c.c. of paraffin-stimulated saliva were collected in sterile flasks from forty-three patients referred by local practitioners for dietary control of dental caries, and from eight members of the staff of the Nutrition Clinic, each of whom had clinical and laboratory evidence of recent dental caries activity. Nine of the patients submitted two samples each.

To determine the comparative effect of the ten selected nitrofuran compounds on acid production, each was added in amounts of 500 micrograms per 10 c.c. of saliva. The number of saliva samples to which each of the test compounds was added is shown in Fig. 3. The amounts of soluble calcium recovered

*At the request of the authors, the nitrofurans employed in these studies were selected and supplied by Dr. W. B. Stillman and Dr. L. E. Daily of the Eaton Laboratories.

from the aliquots containing the nitrofuran compounds were compared with those obtained from untreated aliquots of the same saliva samples which served as the controls. This method has been described in detail in a previous publication.[8]

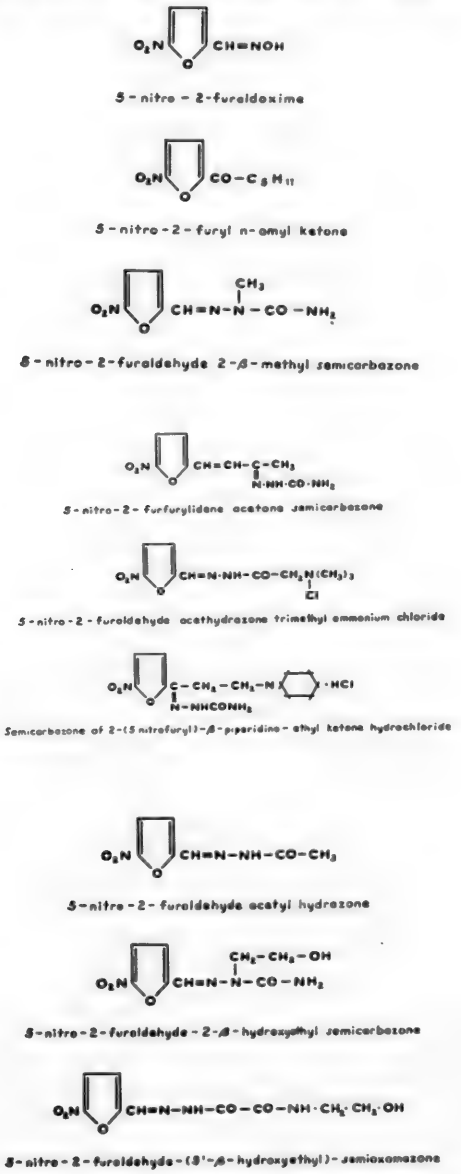

5 - nitro - 2 - furaldoxime
5 - nitro - 2 - furyl n - amyl ketone
5 - nitro - 2 - furaldehyde 2 - β - methyl semicarbazone
5 - nitro - 2 - furfurylidene acetone semicarbazone
5 - nitro - 2 - furaldehyde acethydrazone trimethyl ammonium chloride
Semicarbazone of 2 - (5 nitrofuryl) - β - piperidino - ethyl ketone hydrochloride
5 - nitro - 2 - furaldehyde acetyl hydrazone
5 - nitro - 2 - furaldehyde - 2 - β - hydroxyethyl semicarbazone
5 - nitro - 2 - furaldehyde - (3' - β - hydroxyethyl) - semioxamazone

Fig. 2.—The selected nitrofurans were all related to Furacin but differed from it in the chemical composition of the side chain on the furan ring.

To determine the comparative effect of the selected nitrofurans on the growth and acid production of a pure culture of a strain of the *L. acidophilus*

recovered from a carious lesion, 10 series of tubes were inoculated with a washed suspension of a 24-hour culture of the test organism (*Lactobacillus acidophilus* "Hadley," American Type Culture Collection #4646). Each tube in each series contained 10 c.c. of yeast-extract dextrose broth, one drop of inoculum, and the test compound in an amount ranging from 0.5 mg. to 5.0 mg. per tube. The manner of the preparation of the inoculum and of the yeast-extract dextrose broth which served as the special medium has been presented in detail in a previous publication.[8] Each series was set up in triplicate and incubated at 37° Centigrade. Readings of growth and acid production were taken at the end of 24, 48, and 72 hours. Growth was determined by centrifuging the cultures in Hopkins vaccine tubes at high speed for 10 minutes and then immediately measuring the packed cell volume. Acid production was determined by titrating the centrifugates with 0.1N NaOH to the pH of the control tubes. A Beckman pH meter was used for these determinations.

OBSERVATIONS

The Action of the Test Compounds on Acid Production in Saliva.—The extent to which the addition of 500-microgram quantities of each of the test compounds affected acid production in the saliva is shown in Figure 3. Only those saliva samples in which caries activity was indicated in the control aliquots are included in this tabulation. In no instance did the presence of the nitrofurans alter the results when the control aliquots proved to be caries inactive.

Furacin (5-nitro-2-furaldehyde semicarbazone), in amounts of 500 micrograms, completely inhibited acid production in 65.5 per cent of the caries-active samples, a finding which compares very favorably with the 64 per cent obtained in the preliminary studies. On a comparative basis, it was noted that 6 of the nitrofurans were more effective than Furacin as inhibitors of acid production in the saliva, while 3 were less effective when used in the same amounts per unit of weight. The following percentages, based on the total number of saliva samples rendered negative by the addition of each of the test compounds, indicate the extent to which each of the selected nitrofurans inhibited acid production in the saliva, when added in 500-microgram quantities:

5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone	90.5 per cent
5-nitro-2-furaldehyde-2-B-methyl semicarbazone	85.0 per cent
5-nitro-2-furaldoxime	81.3 per cent
The semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride	75.0 per cent
5-nitro-2-furyl n-amyl ketone	68.8 per cent
5-nitro-2-furaldehyde acetyl hydrazone	66.7 per cent
5-nitro-2-furaldehyde semicarbazone	65.5 per cent
5-nitro-2-furaldehyde-(5'-B-hydroxyethyl)-semioxamazone	53.3 per cent
5-nitro-2-furfurylidene acetone semicarbazone	33.3 per cent
5-nitro-2-furaldehyde acethydrazone trimethyl ammonium chloride	25.0 per cent

It is of interest to note that the test compounds significantly reduced the amount of acid formed in 59 of the 61 aliquots in which the inhibition was not complete. These results were obtained, for the most part, in saliva samples submitted by patients with rampant dental caries.

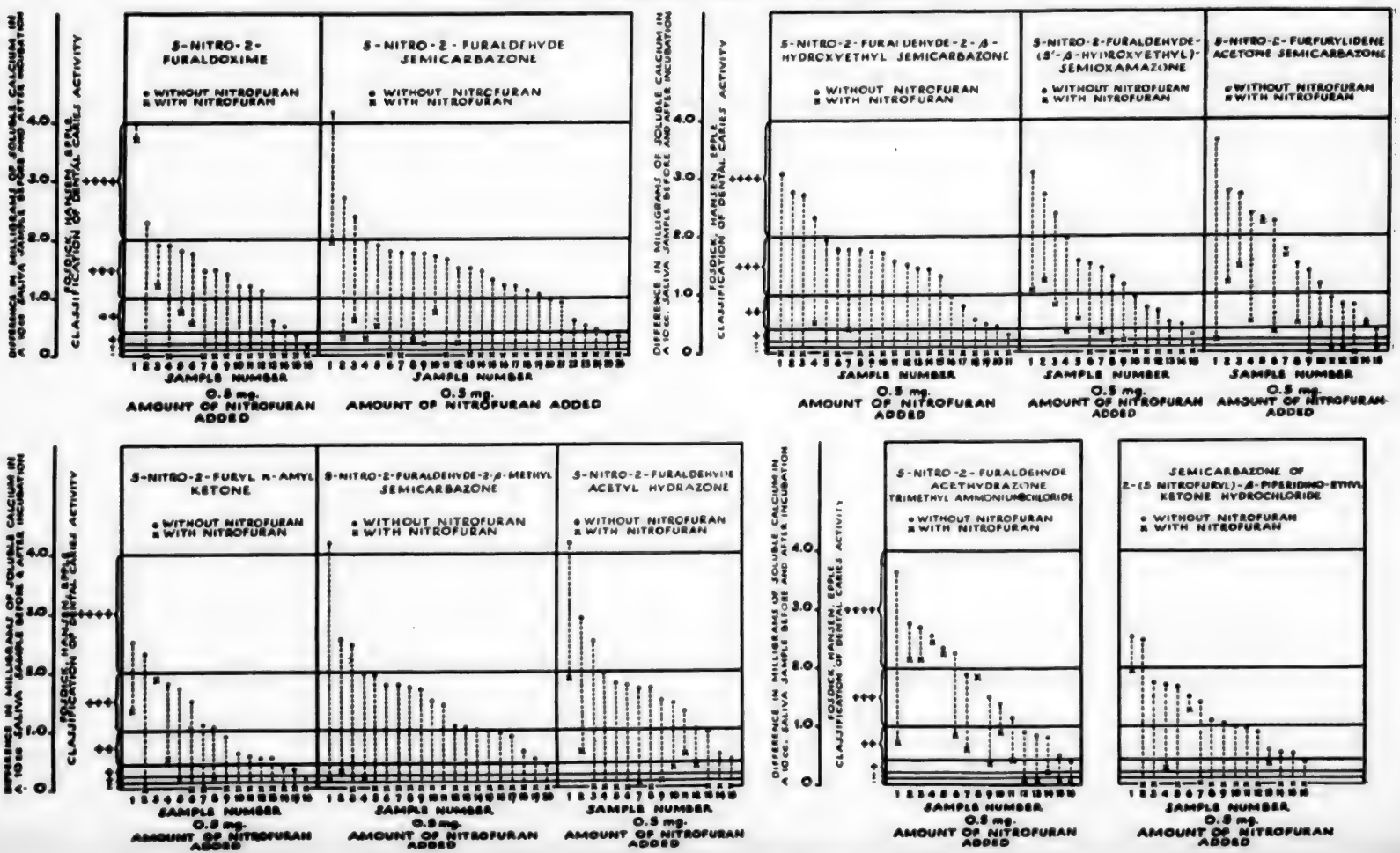


Fig. 3.—*(For legend, see opposite page.)*

The Effect of the Test Compounds on the Growth and Acid Production of the Lactobacillus Acidophilus (Hadley).—The action of the test compounds on the growth and acid production of the test organism is shown in Figure 4. At the end of 24 hours, the growth and acid production of the test organism were completely inhibited in those tubes containing a minimum of 1.0 mg. of 5-nitro-2-furaldoxime, 1.5 mg. of 5-nitro-2-furyl n-amyl ketone, 5-nitro-2-furaldehyde-2-B-methyl semicarbazone, the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride, and 5-nitro-2-furaldehyde-(5′-B-hydroxyethyl)-semioxamazone, 2.0 mg. of 5-nitro-2-furaldehyde-2-B-hydroxethyl semicarbazone, 2.5 mg. of 5-nitro-2-furaldehyde semicarbazone, and 5-nitro-2-furfurylidene acetone semicarbazone, and 3.0 mg. of 5-nitro-2-furaldehyde acethydrazone trimethyl ammonium chloride.

A complete inhibitory effect was observed at the end of 48 hours in those tubes containing a minimum of 2.0 mg. of 5-nitro-2-furyl n-amyl ketone, 2.5 mg. of 5-nitro-2-furaldehyde 2-B-methyl semicarbazone, and 3.0 mg. of 5-nitro-2-furaldehyde-(5′-B-hydroxyethyl)-semioxamazone, 5-nitro-2-furaldoxime, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, and the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride.

At the end of 72 hours, growth and acid production were completely prevented in those tubes containing a minimum of 3.5 mg. of 5-nitro-2-furaldehyde 2-B-methyl semicarbazone, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, 5-nitro-2-furaldehyde-(5′-B-hydroxyethyl)-semioxamazone, and the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride, and 4.0 mg. of 5-nitro-2-furaldoxime.

DISCUSSION

The findings indicate that low concentrations of the ten selected nitrofurans used in these studies will inhibit acid formation in the saliva in vitro. While each of the test compounds was effective in this regard, they differed considerably in their degree of activity when used in the same amount by weight. A similar variation was encountered in the bacteriological studies in which the *L. acidophilus* (Hadley) served as the test organism. The differences in the action of the test compounds may be attributed in part to the differences in the chemical composition of the side chain on the furan ring. This factor appears to affect such properties of the nitrofurans as their solubility in water. The relative inability of some of the test compounds to prevent the growth and acid production of the test organism may be interpreted not only on the basis of differences in solubility but also in the light of reports by Dodd and Stillman[2] and Cramer and Dodd.[9] Dodd and Stillman[2] postulated the existence of specific substances present in broth capable of inhibiting the bacteriocidal and bacteriostatic action of the nitrofuran complex. They based this supposition on their findings that the antibacterial activity of the nitrofurans was enhanced when tested in a synthetic medium. Cramer and Dodd[9] later observed that at low concentrations of Furacin, the lag phase of bacterial growth was prolonged rather than the rate of reproduction being diminished once it was initiated.

Fig. 3.—The sample numbers are arranged in the order of decreasing activity in the control aliquot and not in the order in which the samples were collected. The selected nitrofurans completely prevented acid production in 115 and partially prevented acid production in 59 of the 176 caries-active aliquots tested.

THE EFFECT OF SELECTED NITROFURAN COMPOUNDS
ON THE GROWTH AND ACID PRODUCTION OF THE L. ACIDOPHILUS (HADLEY)

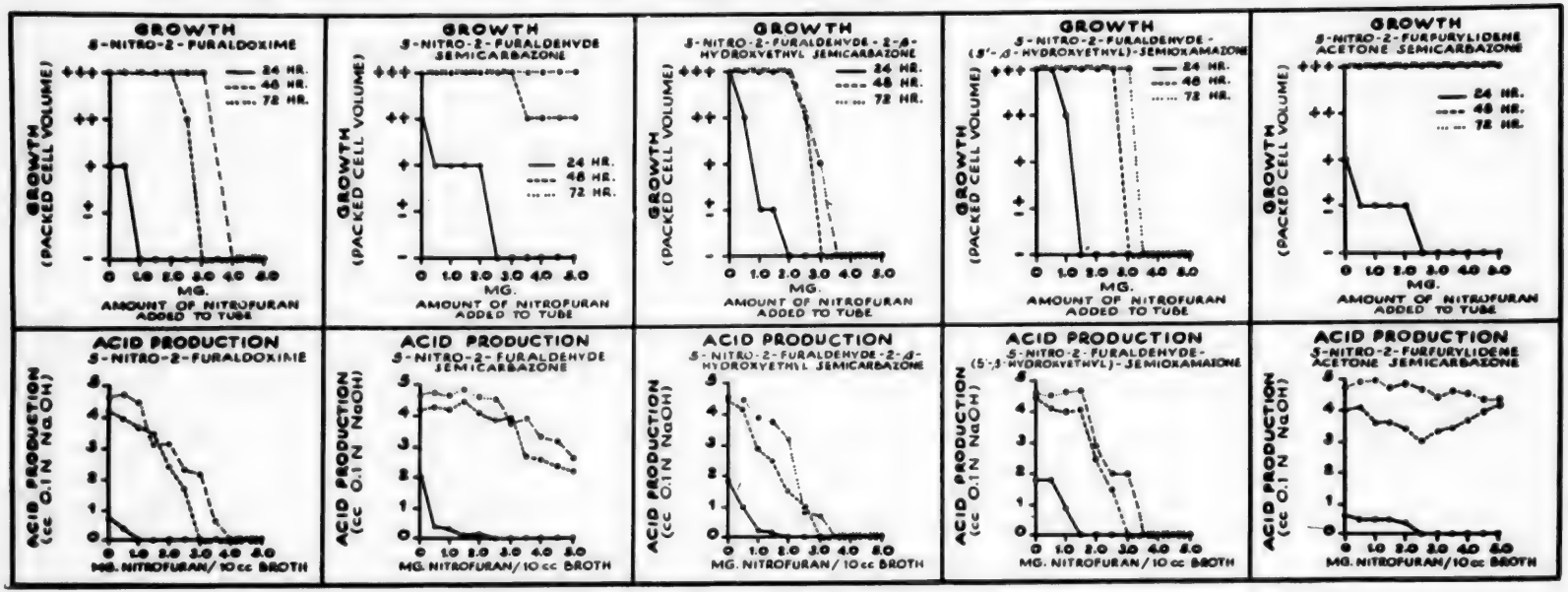


Fig. 4A.—(*For legend, see opposite page.*)

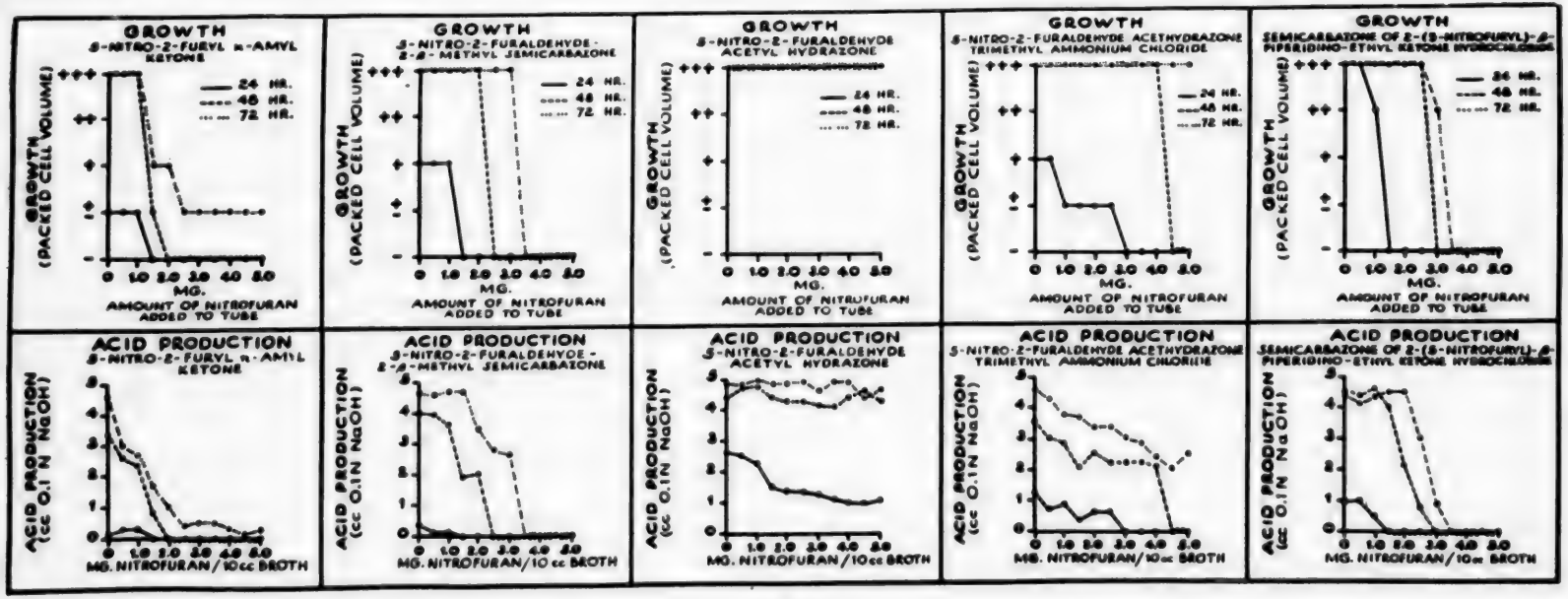


Fig. 4*B*.

Fig. 4.—The amount of growth in each tube was classified as follows:

0.0	- 0.1	c.c.	–
0.1	- 0.25	c.c.	±
0.25	- 0.5	c.c.	+
0.5	- 1.0	c.c.	++
1.0	- 2.0	c.c.	+++

All of the test compounds except 5-nitro-2-furaldehyde acetyl hydrazone completely prevented the growth and acid production of the *L. acidophilus* (Hadley) in yeast-extract dextrose broth at the end of 24 hours in concentrations ranging from 1.0 mg. to 3.0 mg. per 10 c.c. of broth. Five of the test compounds, namely, 5-nitro-2-furaldehyde-2-B-methyl semicarbazone, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, 5-nitro-2-furaldehyde-(5'-B-hydroxyethyl)-semioxamazone, the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride, and 5-nitro-2-furaldoxime, retained their bacteriocidal activity against this organism at the end of 72 hours in concentrations of 3.5 mg. and 4.0 mg. per 10 c.c. of broth.

Greater amounts of each of the test compounds were required to inhibit the growth and acid production of the test organism than were needed to prevent acid production in the saliva.* The explanation may lie in the difference between the buffering capacity of saliva and of yeast-extract dextrose broth; the difference in the time element between the saliva test and the bacteriological test; and the presence of specific substances in broth which interfere with the antibacterial action of the nitrofurans, a factor alluded to by Dodd and Stillman.[2] Saliva is normally a highly buffered biological system containing a significant degree of neutralizing power, whereas the yeast-extract dextrose broth as used in these studies was not buffered. In addition, the organisms in the saliva were exposed to the action of the test compounds for a period of 4 hours, whereas the test organism was exposed to the same compounds for periods ranging from 24 to 72 hours. Dodd and Stillman[2] have shown that several of the nitrofurans are slowly bacteriocidal in high concentrations, while in low concentrations they are merely bacteriostatic for 24 hours or longer.

Several investigators have attempted to explain the antibacterial activity of 5-nitro-2-furaldehyde semicarbazone. These findings may also account for the bacteriocidal and bacteriostatic action of the related nitrofurans used in these studies. Krantz and Evans[6] found that Furacin was capable of diminishing the activity of tissue dehydrogenases, evidence which lends support to an earlier hypothesis by Cramer and Dodd[9] that Furacin may adversely affect some metabolic function of the bacterial cell. Recently, Green[10] suggested that 5-nitro-2-furaldehyde semicarbazone may act by preventing susceptible microorganisms from dissimilating glucose and related substances essential to their nutrition by interfering with their ability to utilize some of the B complex vitamins, notably thiamine and nicotinic acid. Both thiamine and nicotinic acid are essential for the maximum growth of the oral *L. acidophilus*, the organism which plays a large part in the caries process, as well as for the complete degradation of glucose to lactic acid, the acid responsible for the dissolution of the enamel.[11, 12]

SUMMARY AND CONCLUSIONS

1. Ten selected nitrofuran compounds were used in this study. They were 5-nitro-2-furaldehyde semicarbazone, 5-nitro-2-furaldoxime, 5-nitro-2-furyl n-amyl ketone, 5-nitro-2-furaldehyde-2-B-methyl semicarbazone, 5-nitro-2-furaldehyde acetyl hydrazone, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, 5-nitro-2-furaldehyde-(5′-B-hydroxyethyl)-semioxamazone, 5-nitro-2-furfurylidene acetone semicarbazone, 5-nitro-2-furaldehyde acethydrazone trimethyl ammonium chloride, and the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride.

2. These compounds, when added separately in 500-microgram quantities, partially or completely prevented acid production in the saliva, in vitro, in 174 of the 176 caries-active aliquots tested. The aliquots were obtained from 60 samples of saliva submitted by individuals with active dental caries. The most

*It is of interest to note that yeasts were recovered from most of the saliva samples in which 500 microgram quantities of the test compounds were unable to completely inhibit the formation of acid. Additional studies are being made of this aspect of the problem.

efficient inhibitors of acid production in the saliva among the test compounds were 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-B-methyl semicarbazone.

3. All of the test compounds except 5-nitro-2-furaldehyde acetyl hydrazone completely prevented the growth and acid production of the *L. acidophilus* (Hadley) in yeast-extract dextrose broth at the end of 24 hours in concentrations ranging from 1.0 mg. to 3.0 mg. per 10 c.c. of broth. Five of the test compounds, namely, 5-nitro-2-furaldehyde-2-B-methyl semicarbazone, 5-nitro-2-furaldehyde-2-B-hydroxyethyl semicarbazone, 5-nitro-2-furaldehyde-(5′-B-hydroxyethyl)-semioxamazone, the semicarbazone of 2-(5 nitrofuryl)-B-piperidino-ethyl ketone hydrochloride, and 5-nitro-2-furaldoxime, retained their bacteriocidal activity against this organism at the end of 72 hours in concentrations of 3.5 mg. and 4.0 mg. per 10 c.c. of broth.

4. Though further studies are now in progress, these in vitro findings support our working hypothesis that the nitrofurans may prove of value in the control of dental caries because of their pronounced antibacterial action in low concentrations.

We wish to thank Miss Thelma Skinner for her aid in making the caries activity determinations, and Miss Bess Carson for her aid in making the bacteriological determinations.

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DENTAL EFFECTS OF EXPOSURE TO FLUORIDE-BEARING DAKOTA SANDSTONE WATERS AT VARIOUS AGES AND FOR VARIOUS LENGTHS OF TIME

I. Status of the Permanent Teeth of 339 Children Aged 11 to 15 Years Who Used Such Water for Eighteen Months Prior to Eruption of the First Permanent Molars

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IT IS now generally conceded that children, 12 to 14 years of age, who have been exposed to fluoride-bearing communal waters during their entire lifetimes have a more favorable dental caries experience than individuals of the same age who have always lived in areas where the community water is fluoride-free.[1] This fact, coupled with others elicited through experimental work with animals, led to the working hypothesis that blood-borne fluorides deposited in the enamel of a tooth during its calcification were primarily responsible for the caries-inhibitory effect and that the period of fluoride exposure essential to the inhibition of dental caries was that from birth to the age of 8 years.[2]

More recently, Klein has reported upon two groups of individuals who had migrated into a fluoride area after reaching the age of 8 years.[3-6] In both instances marked inhibition of dental caries was observed in teeth which had calcified during residence in a fluoride-free area but which had been exposed to a fluoride-bearing water after eruption. The children were resident in the fluoride area at the time of examination.

The present study is the counterpart of those cited in that it deals with children, residents of a low-fluoride area at the time of examination, who had been exposed to a fluoride-bearing water for varying lengths of time, both prior to and subsequent to the eruption of their first permanent molars.

The present report details the status of the permanent teeth of 873 continuous-residence children of a community which accidentally fluorinated its water for a period of eighteen months extending from August 26, 1935, to March 10, 1937, and in particular with the 339 children now 11 to 15 years of age who received this dosage of fluorides prior to the eruption of their first permanent molars.

All of the children described in this report were residents of Mitchell, a community of approximately 11,000 people situated near the center of the so-called "artesian basin" of South Dakota. This basin extends roughly from the Missouri River eastward to within about thirty miles of the state's eastern boundary, north into North Dakota, and south into Nebraska. In this area surface water tends to be scanty or very hard. Both individuals and communities have drilled artesian wells into the Dakota sandstone to obtain a soft and

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potable water; in some counties of the basin, as many as 80 per cent of the rural wells are artesian wells.[7] Water from this aquifer is remarkably nonfluctuant in its chemical composition, both from place to place and from time to time. Its outstanding characteristics are a high sulfate content and a fluoride level which rises gradually from about 1.5 p.p.m. at the southern edge of the basin to a value of 4.0 p.p.m. or higher at the northern boundary of the state. In this area no well into a sandstone aquifer has ever been reported as yielding a fluoride-free water. Fluorosis in children of this area was studied by Dean, Elvove, and Poston in 1938,[8] and examination reports of children of the basin were the raw data for Dean's first correlation between fluoride exposure and the inhibition of dental caries.[9] Most of the artesian wells within a radius of thirty miles of Mitchell yield water carrying from 2.6 to 3.0 p.p.m. of fluorides.

Prior to August 11, 1930, Mitchell obtained its community water from a number of artesian wells, which were marked as typical Dakota sandstone wells by the high sulfate content of their waters. On that date the city began the use of impounded surface water which has been tested for fluorides periodically since August, 1937; the mean and most constant determination has been 0.2 p.p.m., with comparatively little fluctuation.

In 1935 drought had so depleted this surface supply that "it became necessary . . . to supplement the lake water with well water, and then later it appeared advisable to abandon the lake for water entirely."[10] On August 26 of that year, water from one of the previously used artesian wells, which had been retained as a stand-by source, was turned into the city mains.

Two wells, each approximately 538 feet deep and within a few yards of each other, were used singly or in combination during the following eighteen months. The aquifer is a sandstone which was entered at a depth of 515 feet. In 1946 the wells were reopened for the purpose of obtaining a water sample for fluoride analysis; this sample showed a fluoride inclusion of 2.8 p.p.m., quite consistent with year-by-year analyses of similar wells in the neighborhood. This presumptive evidence that water from these wells contained about that concentration of fluorides during the 1935-1937 pumping period is supported by the observation that about seven per cent of Mitchell teen-age children who used this water during that time now exhibit very mild or mild dental fluorosis.

Pumpage from the wells was intermittent during this period of eighteen months. For the first four months the proportion was about one-third well water to two-thirds lake water. For the following two months well water was used exclusively. During March, April, May, and June of 1936, surface water was used exclusively. Water was then mixed for five months; and for the final three months of this period, well water was again used exclusively. The probable fluoride levels for the period in question, then, were: during the initial four months, about 1.0 p.p.m.; during the following two months, about 2.8 p.p.m.; during the following four months, about 0.2 p.p.m.; during the following five months, slightly above 1.0 p.p.m.; and for the final three months, about 2.8 parts per million. During the entire period 417,702,000 gallons of water (63.54 per cent) were pumped from the low-fluoride surface source, and 239,704,000 gallons

from the wells. Ignoring the variations in mixing the mean fluoride content of Mitchell's tap water for this period was probably in the neighborhood of 1.15 parts per million.

Since March 10, 1937, none of Mitchell's artesian wells has been pumped for more than an hour or two at a time.

In all, 2,140 white children ranging in age from 5 to 22 years were examined, using mouth mirror and explorer. Of this number, 873 were subsequently found to be continuous residents. Among the migrants an additional group of 297 children were found who had been exposed to fluoride-bearing waters somewhere in the South Dakota artesian basin for a period of one year or more before moving to Mitchell.

While the dentists of the area had made extensive use of topical sodium fluoride treatments during the summer before the examinations were begun, it is believed that these treatments were too recent to have made any essential difference in the D.M.F. patterns seen in these children.

METHOD

All of the examinations were made by the author, using No. 5 plane mouth mirrors and explorers, and all were recorded by the same assistant. The same portable operating light was used throughout. The residence history of none of the children was known at the time of examination. The examinations were begun in November, 1947, and concluded in March, 1948. Children of all five elementary schools, the junior high school, and both high schools were included; all but 33 of the children enrolled in the entire school systems were examined.

Criteria were the same as those established for the 1933-1934 national survey[11] except that "catches" were not considered carious in the absence of other indications of disease. An attempt was made to use criteria comparable to those followed during the Hagerstown studies,[12] since comparison with those results was contemplated.

Residence histories for children in the elementary schools were obtained through schedules completed and signed by parents. In the few cases where schedules were not returned, the children were considered to be noncontinuous residents. In the junior and senior high schools the purpose of the survey was explained to the pupils at a preliminary assembly. The importance of an accurate history of residence and water usage was stressed; each child was asked to verify his own history with his parents and to come to the examination room prepared to answer questions in detail. The history was then elicited by interview at the conclusion of each individual examination. The cooperation received was excellent and it is believed that the individual histories so obtained are reasonably accurate and complete. At the outset, random checks were made against original records of Davison County or the State of South Dakota, or individual parents were interviewed for confirmation. Since these checks uniformly supported the history as given by the child this procedure was abandoned after the first 400 children had been examined.

The status of the permanent teeth of the entire group of 2,140 children is outlined in Table I. Their over-all D.M.F. rates are shown graphically in Fig. 1. For purposes of comparison the Hagerstown D.M.F. rate[13] is plotted in Fig. 1, together with a base line previously computed here as representative of the D.M.F. prevalence to be expected in any low-fluoride South Dakota community. This base line is a composite of the examination results for 8,330 children of known low-fluoride areas in 1933-1934,[11] 2,185 in 1946, and 3,206 in 1947, expressed as a curvilinear trend having the formula $Y = 8.75 + 42.43\ X + 3.06\ X^2$.

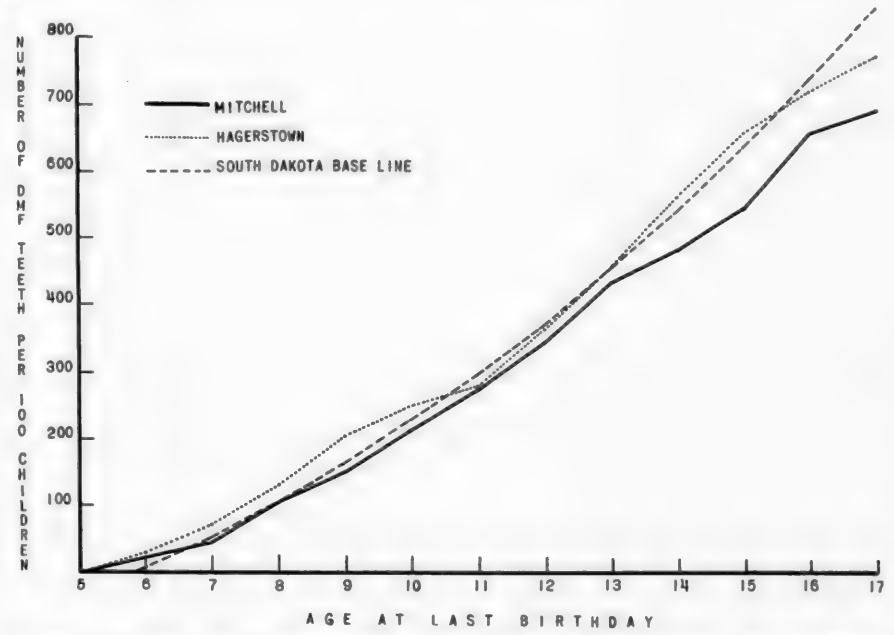

Fig. 1.—D.M.F. rates of 2,094 white elementary and high school children of Mitchell, South Dakota, 1947-1948, together with D.M.F. rates of Hagerstown, Maryland, children and a base line representing age-specific D.M.F. rates expected in South Dakota children who are residents of a low-fluoride community.

It will be seen that, on the whole, the caries experience of Mitchell children is slightly better than either the Hagerstown or the expected experience at all ages, and particularly in children 14 years of age or older; that it tends to follow the pattern of the Hagerstown and basic South Dakota rates, particularly at the younger ages; and that, up through the age of 16, about the same conclusion will be reached regardless of whether a specific Hagerstown rate or a South Dakota base-line rate is used as a basis for comparison.

Continuous-Residence Children.—The term "continuous resident," used for convenience throughout this study, is applied only to Mitchell children who have used Mitchell's community water continuously since birth; certain residents of the city who had used water from other sources during a part of their lives have been excluded from this category. Included in this group are 437 boys

TABLE I
STATUS OF THE PERMANENT TEETH OF 2,140 WHITE ELEMENTARY AND HIGH SCHOOL CHILDREN OF MITCHELL, SOUTH DAKOTA (1947-1948)

AGE GROUP	NUMBER OF CHILDREN	MEAN NUMBER TEETH IN ERUPTION	PER CENT CHILDREN WITH D.M.F. TEETH	NUMBER OF D.M.F.		D.M.F. RATE	MISSING TEETH PER 100 CHILDREN
				TEETH	SURFACES		
5	139	1.25	0.0	0	0	0.0	—
6	156	5.51	11.5	37	42	23.7	—
7	150	8.79	28.0	73	81	48.7	0.7
8	156	11.77	46.2	166	206	106.4	1.3
9	170	13.85	55.3	258	334	151.8	2.9
10	141	18.09	73.0	300	425	212.8	7.1
11	174	21.99	73.0	474	732	272.4	14.4
12	179	25.03	83.2	637	1,072	355.9	30.2
13	161	26.81	84.5	694	1,195	431.1	34.2
14	169	27.59	82.8	809	1,316	478.7	14.2
15	186	27.84	86.6	1,013	1,763	544.6	32.8
16	150	28.12	83.3	990	1,786	660.0	44.7
17	163	28.44	87.7	1,129	2,046	692.6	52.8
18	32	28.63	93.8	223	436	696.9	65.6
19	7	28.43	100.0	107	318	1,528.6	614.3
20	3	31.33	100.0	22	66	733.3	300.0
21	2	29.00	50.0	1	2	50.0	—
22	2	31.00	100.0	9	25	450.0	200.0
Total	2,140		63.2	6,942	11,845		21.8

and 436 girls, a total of 873. The group is further subdivided; there were 216 boys and 209 girls, aged 5 to 10 years, whose fluoride exposure had been negligible, and 165 boys and 174 girls, aged 11 to 15 years, who had been exposed to the accidentally fluorinated water some time after birth and prior to the eruption of the first permanent molars.

The status of the permanent teeth of these children is summarized in Table II and their D.M.F. rates through the age of 15 years are shown graphically in Fig. 2. Continuous-residence children aged 16 years or older are not considered in this report, since their 1935-1937 fluoride exposure came after the eruption of their first permanent molars, and because some of them had previously been exposed to Mitchell's original high-fluoride, artesian-well community water supply.

It will be seen, in Fig. 2, that the rates for these children are somewhat higher than those which were seen in Hagerstown children, or which would have been predicted from the South Dakota base line. Had there been any marked caries-inhibitory effect following the eighteen months of fluoride exposure undergone by the 11- to 15-year age groups, their D.M.F. curve should have broken sharply downward through these age points. This is not the case.

Controls.—Since the expected inhibition of dental caries is absent or negligible, the matter of controls becomes of more than usual importance in this study. There are two standards by which the observed experience of these 11- to 15-year-old children may be judged: their expected caries rates as estimated from the experience of children aged 5 to 10 years now living in the same community, or the actual experience of similar children in other places or at other times.

TABLE II
STATUS OF THE PERMANENT TEETH OF 873 WHITE ELEMENTARY AND HIGH SCHOOL CHILDREN, ALL CONTINUOUS RESIDENTS OF MITCHELL, SOUTH DAKOTA, 1947-1948

AGE GROUP	NUMBER OF CHILDREN	MEAN NUMBER TEETH IN ERUPTION	PER CENT CHILDREN WITH D.M.F. TEETH	NUMBER OF D.M.F.		D.M.F. RATE	MISSING TEETH PER 100 CHILDREN
				TEETH	SURFACES		
5	73	1.47	0.0	0	0	0.0	—
6	68	5.94	13.2	16	17	23.5	—
7	76	8.58	30.3	40	42	52.6	—
8	74	12.14	55.4	94	114	127.0	1.4
9	79	13.84	67.1	151	198	191.1	2.5
10	55	18.58	85.5	147	213	267.3	9.1
11	75	22.01	84.0	246	381	328.0	16.0
12	79	25.35	91.1	307	510	388.6	32.9
13	61	27.05	88.5	318	546	521.3	37.7
14	64	27.59	89.1	353	585	551.6	14.1
15	60	27.95	95.0	457	792	761.7	33.3
16	47	28.15	85.1	313	570	666.0	25.5
17	56	28.32	94.6	428	756	764.3	51.8
18	4	28.75	100.0	32	64	800.0	50.0
19	1	28.00	100.0	2	2	200.0	—
22	1	30.00	100.0	8	24	800.0	400.0
Total	873		65.9	2,912	4,814		16.6

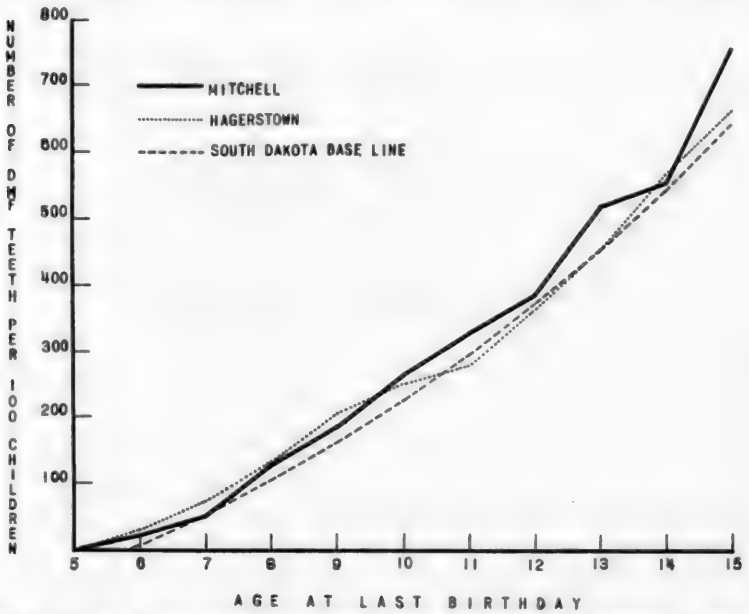

Fig. 2.—D.M.F. rates of 764 white continuous-residence children of Mitchell, South Dakota, 1947-1948, together with D.M.F. rates of Hagerstown, Maryland, children and a base line representing age-specific D.M.F. rates expected in South Dakota children who are residents of a low-fluoride community.

Knutson has demonstrated a rough correlation between the percentages of children with D.M.F. experience at any age and the D.M.F. rate for that age group; he has suggested a mass survey method in which the percentage of children with one or more D.M.F. teeth is obtained for the ages of 7, 9, and 11, from which a straight-line trend is computed and extrapolated as a basis for the

estimation of D.M.F. rates for the older age groups.[14] Had this technic been used at Mitchell, a total of 1,495 D.M.F. teeth would have been estimated for the 339 children aged 11 to 15 years. The total obtained by mouth-mirror examination is 1,680. The Knutson technic, then, would have underestimated the actual number of D.M.F. teeth by 185, an underestimation of 11.0 per cent.

However, with South Dakota data at least, this method invariably underestimates the D.M.F. rates of teen-age children. It presumes that the D.M.F. rate rises with age in a straight line, whereas the actual pattern is usually a pronounced curve. As a matter of fact, age-specific D.M.F. rates for school-age children of either sex plot as a rather complex but typical curve with periods of acceleration and lag which follow periods of tooth eruption and intervals when eruption is at a minimum. When data for school-age groups including both boys and girls are plotted, the peaks and valleys in the sex-specific curves tend to offset each other, the resultant appearing as a fairly smooth parabolic curve with a definite upward acceleration from the age of about 11 years through the teens. This is true of all of the D.M.F. data which have so far come to the author's attention.

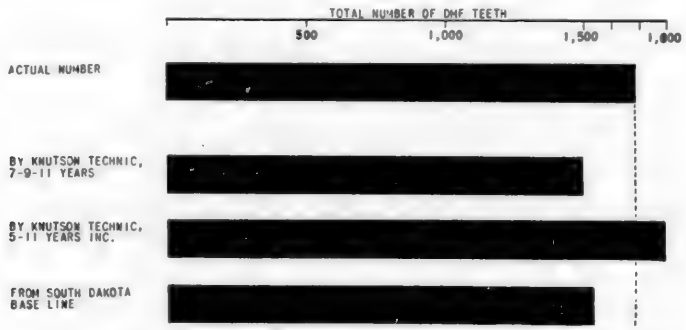

Fig. 3.—Total number of decayed, missing, and filled permanent teeth found in 339 continuous-residence Mitchell, South Dakota, white children, aged 11 to 15 years, and numbers which would have been estimated by various public health technics.

In field practice, then, this division estimates the D.M.F. experience of older children by plotting a parabolic curve which, while still an over-simplification, permits a more accurate prediction of the actual D.M.F. pattern than one based upon a straight-line trend. The percentages of children at all ages up through 11 years are obtained, translated into D.M.F. rates through Knutson's formula, and analyzed as a curvilinear least-squares trend which is then extrapolated into the higher age ranges. Had this method of estimation been used at Mitchell, the total number of D.M.F. teeth in the 11- to 15-year groups would have been computed at 1,790, an overestimation of 110 or 6.58 per cent. In fact, the actual number of D.M.F. teeth would have been underestimated by only 153, or 9.1 per cent by a calculation from the South Dakota base line, even though none of the children at Mitchell had been examined.

The relative magnitude of these totals is shown in Fig. 3.

The first two results pictured are well within the limits of chance fluctuation. The South Dakota base line is not strictly comparable, since it reflects the combined experience of continuous residents and migrants. In the typical South Dakota community a significant percentage of migrant children will have been exposed to a fluoride-bearing water at some time during their lives, and their more favorable caries experience tends to lower the D.M.F. rates for the entire group.

It is clear, then, that eighteen months of fluoride exposure prior to eruption of the first permanent molars either resulted in no inhibition of dental caries in these children, or in one so small that it cannot be demonstrated on the basis of younger but otherwise similar children in the same community, or by comparison with theoretical rates previously computed to be representative of the D.M.F. experience of all present-day children living in low-fluoride communities of South Dakota.

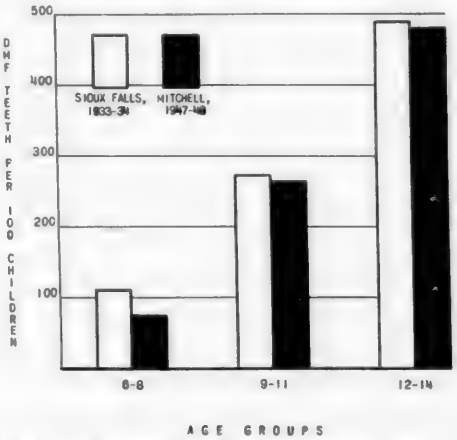

Fig. 4.—Numbers of decayed, missing, and filled permanent teeth per 100 children in Sioux Falls, South Dakota, 1933-1934 (Bulletin 226), and in continuous residents of Mitchell, South Dakota, 1947-1948.

D.M.F. rates for South Dakota children examined in 1933-1934 have been reconstructed from Public Health Bulletin 226[11] by the method used by Dean in his original correlation of South Dakota fluorosis and dental caries data.[9] In general, where no water-supply changes has occurred, these rates approximate very closely those found in South Dakota children of the same communities today. Mitchell children were not examined in 1933-1934, but Bulletin 226 includes data on 1,293 girls and 1,298 boys aged 6 to 14 years who were residents of Sioux Falls, a city 75 miles east of Mitchell and outside the artesian basin, with a kelly-well community water carrying 0.2 p.p.m. of fluorides, the same concentration as that in the present Mitchell water supply.

The data in Bulletin 226 are presented for children in age groups of 6 to 8, 9 to 11, and 12 to 14 years. Similarly grouped data from Table II yield D.M.F. rates for continuous-residence Mitchell children which are compared with those seen in Sioux Falls in 1933-1934 in Table III and in Fig. 4. Inspection of Fig. 4

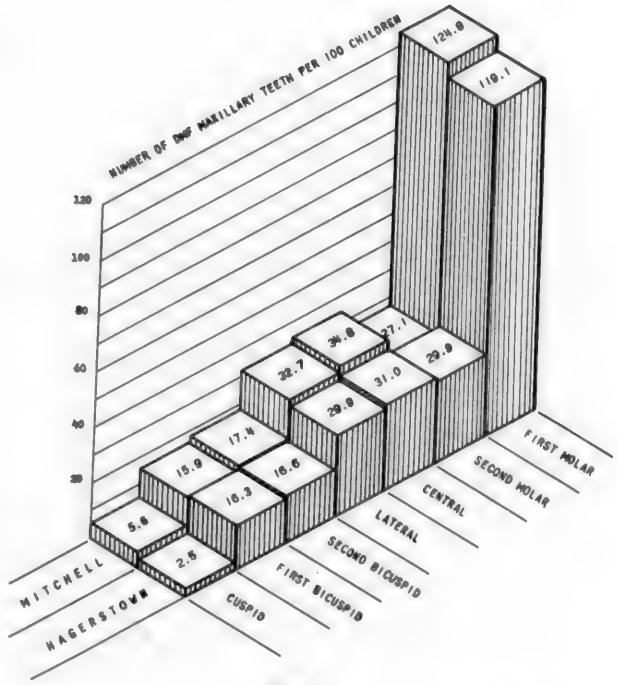

Fig. 5.

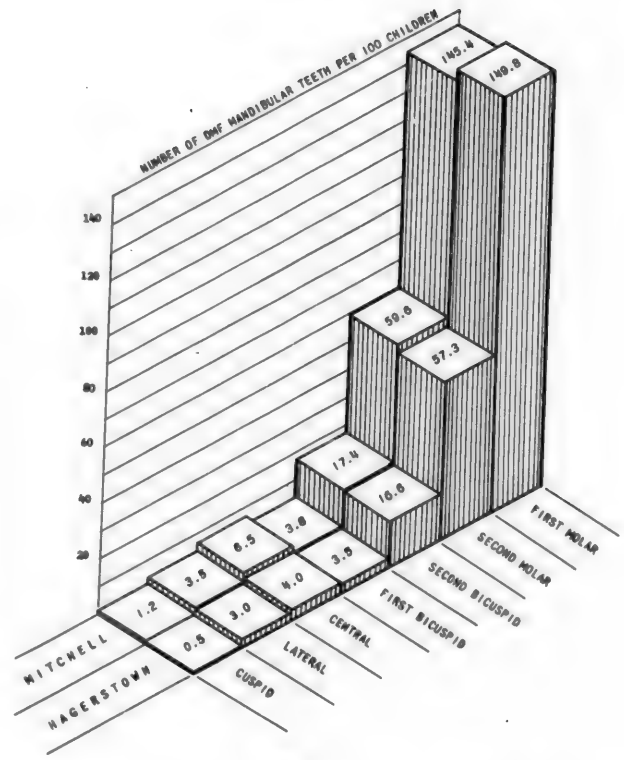

Fig. 6.

Fig. 5.—D.M.F. rates for individual maxillary tooth types in 339 Mitchell, South Dakota, continuous-residence children, aged 11 to 15 years, and for 3,038 Hagerstown, Maryland, children, aged 11 to 15 years.

Fig. 6.—D.M.F. rates for individual mandibular tooth types in 339 Mitchell, South Dakota, continuous-residence children, aged 11 to 15 years, and in 3,038 Hagerstown, Maryland, children, aged 11 to 15 years.

shows that the experience of these two groups of children is substantially the same. The discrepancy in the 6- to 8-year group is probably due to the inclusion of "catches" as caries in the 1933-1934 examination.

TABLE III

D.M.F. RATES AT GROUPED AGES FOR CONTINUOUS-RESIDENCE WHITE CHILDREN OF MITCHELL, SOUTH DAKOTA, 1947-1948, WITH SIMILAR RATES FOR GROUPS OF SIOUX FALLS, SOUTH DAKOTA CHILDREN, 1933-1934

AGE GROUP	MITCHELL			SIOUX FALLS		
	NUMBER OF CHILDREN	NUMBER OF D.M.F. TEETH	D.M.F. RATE	NUMBER OF CHILDREN	NUMBER OF D.M.F. TEETH	D.M.F. RATE
6 to 8	218	150	68.8	1,062	1,149	108.2
9 to 11	209	544	260.3	921	2,465	267.6
12 to 14	204	978	479.4	608	2,985	491.0
Total	631	1,672	265.0	2,591	6,599	254.7

It has been suggested that the caries-inhibitory effect of fluoride exposure is more marked in those morphological tooth types which are normally the more resistant to caries.[6] D.M.F. rates for individual tooth types are available in the Hagerstown data.[15] With the possibility in mind that certain individual tooth types in these Mitchell children might show an inhibition of caries which had been overshadowed in the total rates, the D.M.F. rate was calculated for each morphological tooth type in Mitchell children aged 11 to 15 years, and for Hagerstown children in the same age range. These rates are summarized in Table IV and compared graphically in Figs. 5 and 6.

TABLE IV

D.M.F. RATES FOR INDIVIDUAL TEETH OF 339 WHITE CHILDREN AGED 11 TO 15 YEARS WITH CONTINUOUS RESIDENCE IN MITCHELL, SOUTH DAKOTA (1947-1948) AND OF 3,038 WHITE CHILDREN AGED 11 TO 15 YEARS OF HAGERSTOWN, MARYLAND

TOOTH	MAXILLARY D.M.F. RATES		MANDIBULAR D.M.F. RATES	
	MITCHELL	HAGERSTOWN	MITCHELL	HAGERSTOWN
Second molar	27.1	29.9	59.6	57.3
First molar	124.8	119.1	145.4	149.8
Second bicuspid	17.4	16.6	17.4	13.7
First bicuspid	15.9	16.3	3.8	3.5
Cuspid	5.6	2.5	1.2	.5
Lateral incisor	32.7	29.9	3.5	3.0
Central incisor	34.8	31.0	6.5	4.0
All teeth	258.4	245.2	237.5	231.8

It will be seen by reference to these figures that the two D.M.F. patterns are strikingly similar. Chi square estimation indicates that the only significant differences occur in the upper cuspid and in the lower central incisor. In each case the fluoride-exposed Mitchell children show a substantially higher D.M.F. rate than did the Hagerstown children.

Prevalence of Fluorosis.—One hundred eighty-eight cases of dental fluorosis were discovered in the 2,140 children examined. One hundred fifty-six of these cases occurred in migrant children, appearing as early as the age of 6 and running the entire range of severity. The remaining 32 cases, all very mild or mild in degree, were found in continuous-residence children. Thirty-one

were in children 11 years of age or older, with 26 in the 11- to 15-year group with no known fluoride exposure except that in 1935-1937. The over-all prevalence for these ages was 7.67 per cent. Eight cases were found in 165 boys, a prevalence of 4.85 per cent, and 18 in 174 girls, a prevalence of 10.34 per cent. This variation verges upon statistical significance; the difference is 5.49 ± 2.84, establishing odds against chance of approximately 14 to 1.

SUMMARY AND CONCLUSIONS

1. The community water of Mitchell, South Dakota, was accidentally fluorinated to a mean level of 1.15 p.p.m. for the eighteen months subsequent to August 26, 1936. The normal fluoride content of Mitchell's community water is 0.2 part per million.

2. Of 339 Mitchell children now 11 to 15 years of age who underwent this fluoride exposure prior to the eruption of their first permanent molars, about 10 per cent of the girls and about five per cent of the boys show very mild or mild dental fluorosis.

3. No inhibition of dental caries can be demonstrated in this group on the basis of the caries prevalence in younger children in the same community.

4. No dental caries inhibition can be demonstrated in this group by comparison with a base line calculated from the D.M.F. rates of contemporary children in other South Dakota communities.

5. No caries inhibition can be demonstrated in this group by comparison with the D.M.F. rates of similar children in a comparable South Dakota community in 1933-1934.

6. No inhibition of dental caries can be demonstrated in this group by comparison with over-all D.M.F. rates of Hagerstown children of the same ages, nor by comparison of the D.M.F. rates for individual tooth types between this group and Hagerstown children of the same ages.

7. These findings tend to weaken the hypothesis that the mechanism whereby dental caries is inhibited through exposure to fluoride-bearing drinking waters depends essentially upon the deposition of fluorine in dental enamel during the period of calcification.

The sincere thanks of the author are due to Superintendent of Schools Lloyd Uecker, Sister M. Martha, City Engineer J. C. Scallin, and in particular to School Nurse Helene Tobin Baker for their unfailing courtesy and their essential assistance during the fourteen weeks this survey was in progress in the Mitchell schools.

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FLUORIDE SATURATION RATE OF HUMAN ENAMEL

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IT HAS been adequately demonstrated that a temporary caries-reducing effect is produced by topical treatment of erupted teeth with a fluoride solution. It has been similarly shown, in vitro, that treatment of teeth, of fragments of teeth, of pulverized enamel and dentin with fluorides considerably increases their resistance against acid solvents.[1] It is generally accepted that fluoride ions react chemically with the constituents of erupted enamel and enter the crystallites forming new, fluorosed chemical compounds.[2] If this concept is true, the quantity of fluorides in the solution must be gradually reduced, within certain limits, while the treated teeth or dental hard tissues become saturated with fluorides. An equilibrium must be gradually established between fluoride in solution and the fluoride content of dental tissues. In literature accessible to us, we found no data upon this question. For this reason, we carried out a few experiments, in vitro, upon this problem.

METHOD

Intact parts of enamel of carious teeth that were extracted some years ago, cleansed and kept dry, were used in the experiments. Enamel was separated from dentin with dental burs and hand instruments under macroscopic control. It was then pounded in a mortar to coarse granules. In this manner, a considerable increase of the surface upon what the fluoride solution can act is effected.

For the sake of convenience, in fluorine determination, an aqueous NaF solution with approximately 1 mg. per cent concentration of fluoride ions was used.

Five Gm. coarsely granulated enamel was exposed to the action of 200 ml. of this NaF solution. After the elapse of 10, 20, 30, 45, 60, 90, 120, and 180 minutes, 2 to 12 ml. of the solution was pipetted away for fluorine determination, using the method as described by Fellenberg.[3]

In samples of enamel, fluorine concentration was determined previously to experimentation. Experiments and determinations were arranged in duplicate.

RESULTS AND DISCUSSION

The average of two experimental series is reproduced in Table I. The data of this table indicate that the maximum loss of fluorine in the solution is observed after 60 minutes. If enamel is continually exposed to the fluoride solution, further slight changes in fluorine concentration take place, but there is no continuous increase or decrease. We are prone to regard these changes as being due to dissolution of fluorosed enamel surface layers in distilled water of neutral reaction or in an aqueous NaF solution and to continued reaction of fluorides with deeper enamel layers. In consideration of the slight changes

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that are taking place after the first hour, it can be taken for granted that consumption of fluorine by the enamel is influenced too by processes of dissolution. The fluorine loss of the solution as determined in the experiments may be regarded as the difference in the quantity of fluoride ions that entered the enamel crystallites and of the small amount that gradually becomes dissolved again.

TABLE I

FLUORIDE CONSUMPTION OF 5 GM. COARSELY GRANULATED HUMAN ENAMEL, CONTAINING ORIGINALLY 0.380 MG. FLUORINE

TIME IN MINUTES	FLUORINE CONTENT OF 200 ML. NaF SOLUTION (MG.)	QUANTITY OF FLUORINE LOST FROM THE SOLUTION (MG.)
0	2,040	–
10	1,820	0,220
20	1,495	0,545
30	1,300	0,740
45	1,038	1,002
60	*0,671*	*1,369*
90	0,734	1,306
120	0,739	1,301
150	0,726	1,314
180	0,720	1,320

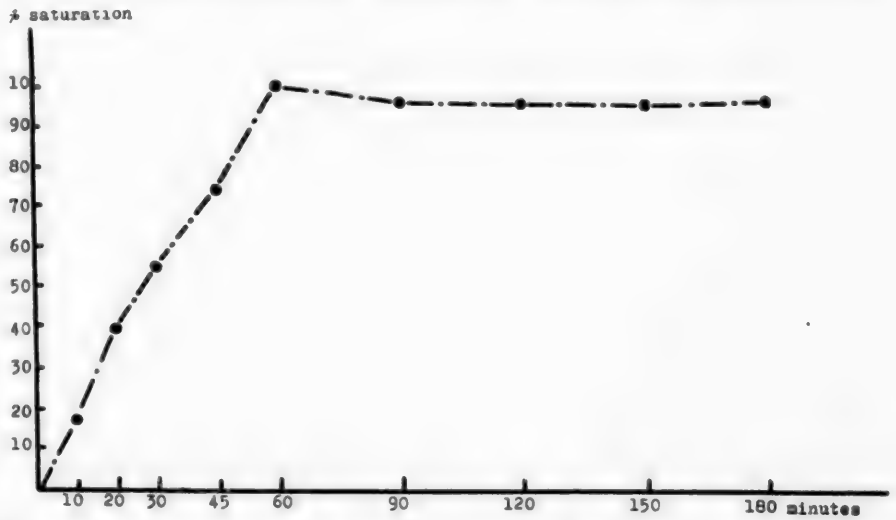

Fig. 1.—Fluoride saturation rate of 5 Gm. coarsely granulated human enamel exposed to 200 ml. of an aqueous NaF solution with 1 mg. per cent F ions.

In evaluating the data of experiments, fluorine consumption of coarsely granulated enamel is best expressed in per cents of complete saturation (Fig. 1). With reference to complete saturation, it was essential to clarify whether by consumption of 1.369 mg. fluorine, 5 Gm. coarsely granulated enamel attained full saturation or only an equilibrium valid under prevailing special conditions. At the stage of maximal saturation, 0.671 mg., fluorine remained dissolved in the solution.

At what rate saturation occurs if 5 Gm. of enamel is exposed to 100 ml. of the NaF solution, containing originally a total of 1.03 mg. fluorine, was determined. Maximal saturation is attained after the elapse of 2 hours, but in 60 minutes 95.37 per cent of this has been attained already. Maximal saturation caused a drop of fluorine in the solution from 1.03 mg. to 0.167 milligrams.

From this experiment, we may see that enamel may reduce the fluorine content of the NaF solution far below 0.671 mg. while reaction between enamel and fluoride is still taking place. We can thus conclude that if, in the experiments shown by the data in Table I, the saturation were not complete, chemical reactions could be continued until the drop of F ions in the solution reached 0.167 instead of to 0.671 milligram. This seems indicative that under conditions of the experiment (Table I), complete saturation was attained; using 100 ml. of the NaF solution, on the other hand, only a special equilibrium was established.

It is obvious, a priori, that saturation rate must be dependent upon the original fluorine content of the enamel, upon the concentration of the fluorine solution used, and finally upon the extent of surface where it acts. To clarify these variables is the aim of further study. Results of present experiments indicate that saturation with very dilute solutions occurs within a relatively short time.

Stress is to be laid upon the observation that in 20 minutes approximately 40 per cent saturation is attained and, thereby, the fluorine in the enamel becomes more than doubled. This is indicative that short topical treatment with fluorides, after tooth eruption, may have a profound influence upon the chemical structure of enamel surface layers.

A second fact deserves consideration. Enamel with 0.380 mg. of fluorine was saturated by 1.369 mg., i.e., approximately 3.6 times more fluorine than it originally contained. Enamel was taken from teeth selected at random, but they may be regarded as typical for Hungary. The high caries incidence and these experimental results indicate an insufficient chemical structure of enamel, a fluoride deficiency in Hungary. This is in agreement with former studies of the senior author[4] upon fluorides in water supply.

SUMMARY

The fluoride saturation rate of coarsely granulated human enamel was ascertained using an aqueous 1 mg. per cent of NaF solution. Maximum saturation was attained in 60 minutes, but in 20 minutes approximately 40 per cent saturation was attained when 5 Gm. of enamel was exposed to 200 ml. NaF solution. Enamel is capable of saturation with 3.6 times more fluorine than it originally contained.

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STUDIES ON THE NUMBERS OF BACTERIA IN THE MOUTH AND THEIR REDUCTION BY THE USE OF ORAL ANTISEPTICS

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THE problems involved in the determination of the numbers and types of bacteria in the mouth are extremely difficult and complex. No standard technics are available as yet for the quantitative estimation of the oral bacterial flora. Even the quantitative method for estimating lactobacilli in saliva as developed by Hadley[1] and others is subject to considerable error.[2, 3] It is important that more accurate methods for the determination of the numbers of bacteria in the mouth be developed, particularly if oral disinfectants are to be evaluated on the basis of in vivo tests.

In the present paper, attention is given to methods and media for the estimation of the number of bacteria in the mouth. Data are also presented on the number of certain types of bacteria in the mouth and on the bactericidal action of glycerite of hydrogen peroxide, Cepacol, and penicillin troches on the oral bacteria.

METHODS AND NUMBERS OF BACTERIA IN THE MOUTH

Culture Media

The culture media used for cultivating oral bacteria will depend to a large extent on the types of bacteria to be cultivated. In the present study, we were interested in obtaining counts on the general oral flora, oral streptococci, and lactobacilli.

In previous studies reported by the authors,[4] tryptone glucose extract agar was used for estimating numbers of the general or common mouth bacteria. Since this medium offers no information as to the number of viridans or weakly hemolytic streptococci in the mouth, studies were made on the comparative value of a blood agar medium and the tryptone glucose extract agar medium for estimating number of oral bacteria. The latter medium contained 0.5 per cent Difco tryptone, 0.5 per cent Difco beef extract, 0.1 per cent glucose, and 1.5 per cent agar. The blood agar had the following composition: 2 per cent Difco tryptose, 0.1 per cent glucose, 0.5 per cent NaCl, 2 per cent agar, and 5 per cent citrated sheep blood. The reaction of both media was adjusted to pH 7.0.

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Samples for test were collected by a brushing and rinsing technic[4] from the mouths of three subjects at 11:00 A.M. each day over a six-day period. After shaking for ten minutes in a Kahn shaking machine, all samples were plated in the tryptone glucose extract agar and in tryptose glucose blood agar using dilutions of 1:10,000, 1:100,000, and 1:1,000,000. Colonies were counted after incubation of the cultures at 37° C. for two days. The oral streptococci on the blood agar appeared as small colonies surrounded by a slight hemolysis or greening of the blood.

Examples of the results obtained are recorded in Table I. The total counts on the blood agar were as high or higher than those on the tryptone glucose extract agar. Since the blood agar has the added advantage of affording counts on the numbers of streptococci in the mouth, this medium was used for total counts and streptococcus counts throughout the remainder of this study. The total counts listed for the blood agar include colonies of the viridans streptococci. It is interesting to note the large number of these organisms present in samples from the mouth; they always made up a large portion of the total number of bacteria developing on the blood agar.

For the determination of the numbers of lactobacilli, suitable dilutions of the saliva samples were plated in tomato juice agar containing 40 per cent filtered tomato juice, 1 per cent Difco peptone, 1 per cent Difco peptonized milk, and 2 per cent agar, adjusted to pH 5.0 with lactic acid. The cultures were incubated in an atmosphere of carbon dioxide (10 per cent) for two days at 37° Centigrade. Comparative studies indicated that more accurate counts were obtained when pour plates were prepared than when the diluted samples were smeared over the surface of the agar.

As a diluent of the saliva samples, a sterile phosphate buffer solution was used similar to that recommended in the methods for the determination of the number of bacteria on eating utensils. The stock solution contained 34 Gm. of potassium dihydrogen phosphate in 825 ml. of distilled water and 175 ml. of normal NaOH solution, adjusting the mixture to a pH of 7.2. The final diluent is prepared by adding 1 ml. of this solution to 800 ml. of distilled water and sterilizing in the desired quantities.

TABLE I

COMPARISON OF TRYPTONE GLUCOSE EXTRACT AGAR AND BLOOD AGAR FOR THE ESTIMATION OF THE NUMBER OF ORAL BACTERIA

SUBJECT	TRYPTONE AGAR	BLOOD AGAR	
	TOTAL COUNT*	TOTAL COUNT	VIRIDANS STREPTOCOCCI
B	290,000,000	287,300,000	185,800,000
D	173,800,000	191,200,000	115,500,000
S	160,700,000	167,100,000	128,000,000

*All counts represent an average of six tests in samples collected each day for a six-day period.

Methods for the Collection of Samples From the Mouth

The paraffin chewing-saliva collection technic described by Hadley[1] has been used by most investigators for obtaining samples for the estimation of the number of lactobacilli in the mouth. However, in studies on the number of

bacteria in the mouth, various workers have also employed a brushing and/or rinsing technic.[4, 5, 6, 7, 8, 9, 10] A comparison of the numbers of bacteria collected by these two technics seemed advisable.

The brushing, rinsing technic described by Slanetz and Brown[4] was employed in this study. A sterile brush is dipped in 10 ml. of sterile water and the teeth brushed vertically for two minutes. The toothbrush is rinsed in the remainder of the sterile water after one-minute brushing and again at the end of the period. After collecting the saliva in the collection tube, the mouth is then rinsed vigorously for one minute with 10 ml. of sterile water and this rinsing added to the material collected from the toothbrush. The total volume is adjusted to 20 ml., using the phosphate buffer solution as the diluent.

For the paraffin-saliva technic, small squares of paraffin approximately one-half inch in size were chewed for three minutes, and all the saliva secreted during this time was collected in sterile tubes 30 mm. in diameter and 100 mm. in length. The total volume was then adjusted to 20 ml. with the phosphate buffer solution.

Three subjects were used for the tests. Samples were collected at 11:00 A.M. each day for a five-day period by the brushing technic and the following week for the same period by the saliva method. After shaking for ten minutes in a Kahn shaking machine, all samples were plated out in tryptose glucose blood agar using dilutions of 1:10,000, 1:100,000, and 1:1,000,000. The colonies on the plates were counted after incubation at 37° C. for two days.

Some of the results obtained are summarized in Table II. It is evident that many more organisms were collected by the paraffin-saliva technic than by the brushing technic. Since it might be argued that the difference in the numbers could be due to greater numbers of bacteria normally in the mouth during the second week than during the first week of the test, another experiment was carried out. For this test, samples were collected on each of two days from two subjects, first by the brushing technic and one-half hour later by the paraffin-saliva technic. The samples were plated out as previously described and the results are recorded in Table III. It is again evident that many more organisms were collected by the paraffin-saliva technic, the counts being from 39.5 per cent to 59.8 per cent higher than those obtained on samples collected by the brushing technic. Because of this fact and since the paraffin saliva-technic is easier to execute and control, this method was used for the collection of samples for all tests reported in the following sections of this paper.

TABLE II

COMPARISON OF SAMPLING TECHNICS FOR THE ESTIMATION OF NUMBERS OF ORAL BACTERIA

	BRUSHING TECHNIC		PARAFFIN-SALIVA TECHNIC	
SUBJECT	TOTAL COUNT* BLOOD AGAR	VIRIDANS STREPTOCOCCI	TOTAL COUNT BLOOD AGAR	VIRIDANS STREPTOCOCCI
B	260,500,000	170,900,000	396,000,000	319,000,000
D	191,200,000	115,460,000	434,000,000	314,000,000
S	82,416,000	61,383,000	324,000,000	260,000,000

*All counts represent an average of six tests on samples collected each day for a six-day period.

Effect of the Length of the Paraffin-Chewing Period on the Numbers of Bacteria Collected in the Saliva From the Mouth

In the paraffin-saliva technic for determining numbers of bacteria in the mouth, three minutes appears to be accepted as standard for the time during which the paraffin is chewed and the saliva collected. Studies were made to determine how many bacteria are collected during each one-minute chewing period and what effect chewing the paraffin for longer periods would have on the number of bacteria found in the saliva samples.

Tests were made using two subjects. In the first series of tests, the saliva was collected at one-minute intervals in separate vials for a period of five minutes. The saliva obtained for each period was measured and the number of bacteria present per ml. determined by plating out dilutions of 1:100,000 and 1:1,000,000 in the tryptose glucose blood agar. Counts were made on the total number of bacteria developing on the plates after incubation at 37° C. for two days.

An example of the results obtained is shown in Table IV. While there was some variation in the numbers of bacteria found in the samples collected at successive one-minute periods, it was surprising to note the large numbers still present in the samples collected during the four- to the five-minute period. The total counts obtained during the three- and five-minute collection periods are also indicated.

In the second series of tests, saliva samples were collected over a fifteen-minute chewing period. The number of bacteria per ml. of saliva collected

TABLE III

COMPARISON OF SAMPLING TECHNICS FOR THE ESTIMATION OF NUMBERS OF ORAL BACTERIA

SUBJECT	DATE	BRUSHING TECHNIC		PARAFFIN-SALIVA TECHNIC	
		TOTAL COUNT* BLOOD AGAR	VIRIDANS STREPTOCOCCI	TOTAL COUNT BLOOD AGAR	VIRIDANS STREPTOCOCCI
A	4/27	315.6	267.3	532.6	454
A	4/29	1,872.6	1,578.5	4,406.6	3,990
B	4/27	170	160	410	330
B	4/29	501	479	867	800

*Numbers of bacteria are listed in millions. Each count is an average of the numbers on three plates of each dilution.

TABLE IV

NUMBERS OF BACTERIA IN SALIVA SAMPLES COLLECTED AT SUCCESSIVE ONE-MINUTE INTERVALS OVER A PERIOD OF FIVE MINUTES

MINUTE OF CHEWING	SUBJECT R		SUBJECT B	
	TOTAL COUNT PER ML.	VOLUME OF SALIVA IN ML.	TOTAL COUNT PER ML.	VOLUME OF SALIVA IN ML.
First	43,500,000	1.7	18,800,000	3.2
Second	33,900,000	2.4	25,400,000	3.6
Third	8,100,000	2.0	17,900,000	2.4
Fourth	29,000,000	2.1	3,000,000	2.6
Fifth	20,400,000	2.2	22,900,000	2.4

	SUBJECT R	SUBJECT B
Total count after 3 minutes:	171,600,000	194,600,000
Volume of saliva after 3 minutes:	6.1 ml.	9.2 ml.
Total count after 5 minutes:	277,400,000	257,400,000
Volume of saliva after 5 minutes:	10.4 ml.	14.2 ml.

during each minute period was determined for the first, second, fifth, sixth, seventh, eighth, ninth, tenth, and fifteenth minute periods following the method described above. Saliva obtained during the periods not tested was discarded.

Some of the results obtained are listed in Table V. Large numbers of bacteria were found per ml. of saliva up to the tenth and even the fifteenth minute chewing period. The washing out of the bacteria by saliva apparently is a gradual process which is probably influenced some by the continual multiplication of the organisms during the chewing period. Since the volume of saliva collected often decreases after each minute chewing period, the largest total numbers of bacteria are collected in the early chewing periods. On the basis of these studies, it is felt that the collection of saliva over a three-minute period is satisfactory for the estimation of the numbers of bacteria in the mouth. However, we feel that the counts should be based on the total number of bacteria removed and not on the number per ml. of saliva samples. This is particularly important when comparative tests on the efficiency of oral antiseptics are made since subjects vary as to the amount of saliva produced and the same subject may vary in the amount stimulated on different days. Variation in the rate of secretion of saliva has also been demonstrated by Trimble, Etherington, and Losch.[11]

Determination of the Numbers of Bacteria in the Mouth at Different Times of the Day

In studies on the numbers of bacteria in the mouth made in this laboratory, samples were usually collected at 10:00 or 11:00 A.M. each day. Since the numbers of bacteria may fluctuate during different periods of the day, tests were made to determine the numbers of bacteria in the mouth of four subjects at ten different periods during the day on eight different days over a one-month period. Samples were collected by the paraffin-saliva technic at 7:00 A.M. (on arising), 7:30 A.M. (after brushing teeth), 8:00 A.M. (after breakfast), 10:00 A.M., 12:00 A.M. (before lunch), 12:30 P.M. (after lunch), 3:00 P.M., 6:00 P.M. (before dinner), 6:30 P.M. (after dinner), and 11:00 P.M. All samples were plated out in tryptose glucose blood agar and tomato agar.

TABLE V

NUMBERS OF BACTERIA PER ML. OF SALIVA COLLECTED DURING EACH ONE-MINUTE INTERVAL OF PARAFFIN CHEWING OVER A FIFTEEN-MINUTE PERIOD

MINUTE OF CHEWING	SUBJECT D		SUBJECT M	
	TOTAL COUNT PER ML.	VOLUME OF SALIVA IN ML.	TOTAL COUNT PER ML.	VOLUME OF SALIVA IN ML.
First	27,600,000	2.8	9,600,000	3.8
Second	23,500,000	2.4	9,100,000	4.0
Fifth	18,700,000	2.0	10,700,000	2.8
Sixth	19,300,000	2.0	12,100,000	2.2
Seventh	15,800,000	1.7	12,200,000	2.4
Eighth	10,600,000	1.6	14,000,000	2.0
Ninth	6,200,000	1.9	8,100,000	2.0
Tenth	9,100,000	1.7	14,200,000	2.2
Fifteenth	5,300,000	1.3	6,300,000	2.0

While it is difficult to compare and interpret the results obtained, it is evident that there is considerable variation in the number of bacteria found in the mouth during different periods of the day. In general, the greatest numbers were found in the samples collected at 7:00 A.M., 12:00 A.M., and at 11:00 P.M. There was a definite decrease in numbers immediately after meals. Jay[12]

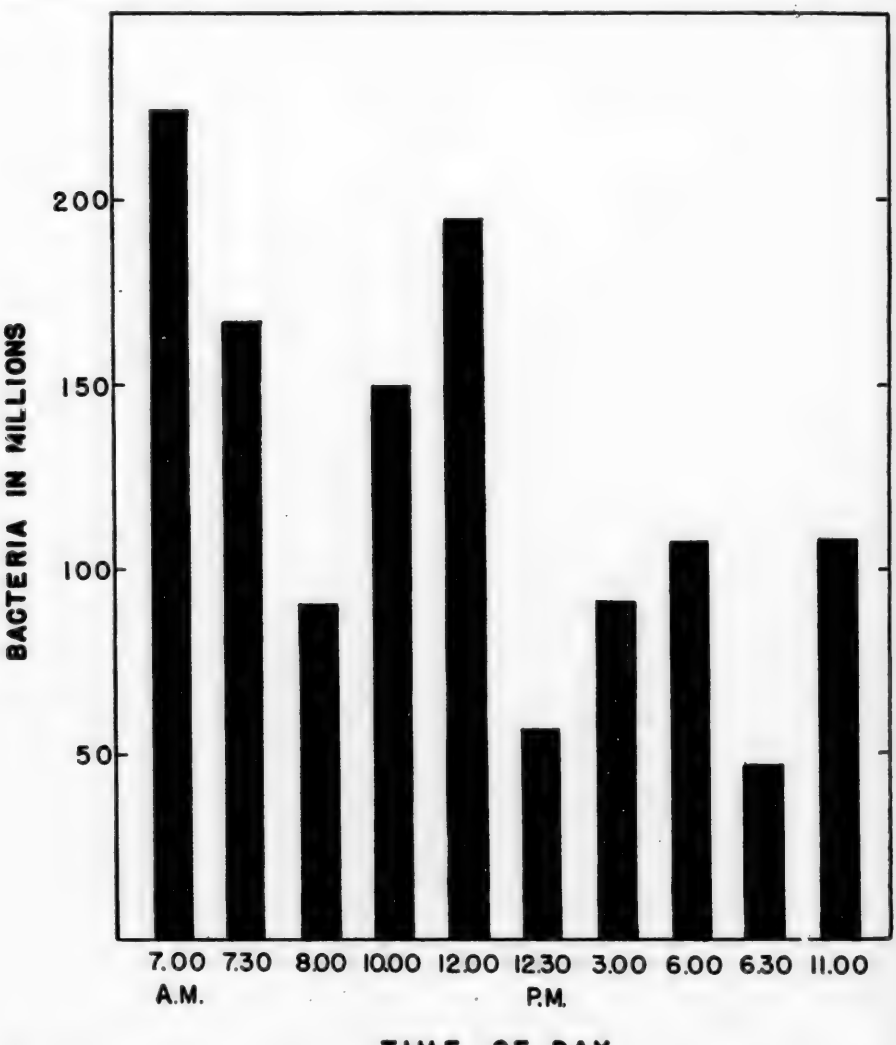

Fig. 1.—Number of bacteria in the mouth at different periods of the day based on total counts on blood agar. Average of eight tests over a one-month period.

has also apparently observed this reaction. As was expected, there was a variation in the number of bacteria in the samples from a given subject taken at the same time on different days. An example of typical results obtained is given for one subject in Fig. 1. The numbers of bacteria represent the total

number of bacteria collected in the saliva samples as determined by plating in blood agar. In general, when there was an increase or decrease in the total count, there was a corresponding change in the number of viridans streptococci and lactobacilli. It is evident that, for comparative studies on the number of bacteria in the mouth, samples should be collected at approximately the same time each day.

BACTERICIDAL ACTION OF ORAL ANTISEPTICS

In a previous paper,[3] data were presented on the bactericidal action of glycerite of hydrogen peroxide and penicillin troches on bacteria in the mouth. Further studies on the action of these agents and on Cepacol are reported in the present paper.

For the determination of the numbers of bacteria in the mouth before and after the use of the test antiseptics, saliva samples were collected by the three-minute paraffin-chewing technic. The saliva samples were adjusted to a total volume of 20 ml. with the phosphate buffer diluent, and suitable dilutions then plated in the tryptose glucose blood agar and the tomato juice agar previously described. Duplicate and triplicate plates of a given dilution were made for many of the samples. All cultures were incubated at 37° C. for two days, the tomato juice agar plates being held in an atmosphere of 10 per cent carbon dioxide. Total counts and counts for viridans or weakly hemolytic streptococci were made on the blood agar. The tomato agar plates afforded counts on the numbers of lactobacilli. All figures reported are based on the total number of bacteria present in the saliva sample collected from each subject.

Examples of the results of these studies using sixteen subjects are recorded in Tables VI, VII, and VIII. The subjects ranged in age from 17 to 45 years and were mostly college students. Six of the subjects rinsed their mouths for one minute with 10 ml. of glycerite of hydrogen peroxide solution* (0.5 per cent) twice daily following the usual morning and evening brushing of the teeth. Another group of five subjects folowed the same procedure but used Cepacol† (undiluted) as the rinsing agent. The remaining five subjects dissolved three penicillin troches‡ (5000 units) in their mouths daily, one after each meal.

Saliva samples were collected at 10:00 A.M. each day over five-day periods before, during, and after medication. Table VI indicates the total counts on blood agar before and after medication. Table VII gives counts of oral streptococci and Table VIII, numbers of lactobacilli. In considering these results, it should be pointed out that all counts are based on samples collected at 10:00 A.M. each day and not immediately after the disinfectants were used. Definite reductions were obtained in the numbers of bacteria in the mouth following the use of the test antiseptics in most of the subjects. For the glycerite of hydrogen peroxide the reduction in the total count on blood agar, the

*The glycerite of hydrogen peroxide (1.5 per cent solution) was supplied through the courtesy of the International Pharmaceutical Corp., Boston, Mass.

†The Cepacol was supplied through the courtesy of Wm. S. Merrell Co., Cincinnati, Ohio.

‡The penicillin was supplied through the courtesy of Lederle Laboratories, Pearl River, N. Y.

viridans streptococci and the lactobacilli averaged from 24 to 87 per cent, 18 to 96 per cent, and 15 to 86 per cent respectively. For the Cepacol, the reduction averaged 4 to 68 per cent, 0 to 89 per cent, and 0 to 74 per cent respectively. Very marked diminution in the oral flora was obtained with the penicillin troches. This was particularly pronounced for the oral streptococci which were reduced to very small numbers in the five subjects tested. This

TABLE VI

REDUCTION IN NUMBER OF ORAL BACTERIA FOLLOWING DAILY USE OF TEST MEDICATIONS FOR A FIVE-DAY PERIOD

(Numbers listed in millions. Average of five tests)

MEDICATION	SUBJECT	TOTAL COUNT BEFORE	TOTAL COUNT AFTER	PER CENT DECREASE
Glycerite of hydrogen peroxide (0.5 per cent or 1:2 concentration), two rinses daily	R	411	62	85
	4	1030	784	24
	L	129	16	87
	St	88	11	87
	Sc	86	31	64
	1	875	410	53
Cepacol (undiluted), two rinses daily	11	1090	1044	4
	12	820	640	22
	13	1360	432	68
	14	760	564	26
	Re	411	160	61
Penicillin troches (5,000 units), three daily	6	1656	6	99.6
	7	796	19	97
	8	792	21	97
	9	1596	5	99.7
	10	763	3	99.9

TABLE VII

REDUCTION IN NUMBER OF ORAL STREPTOCOCCI FOLLOWING DAILY USE OF TEST MEDICATIONS FOR A FIVE-DAY PERIOD

(Numbers listed in millions. Average of five tests)

MEDICATION	SUBJECT	ORAL STREPTOCOCCI BEFORE	ORAL STREPTOCOCCI AFTER	PER CENT DECREASE
Glycerite of hydrogen peroxide (0.5 per cent or 1:2 concentration), two rinses daily	R	297	11.3	96
	4	848	69.2	18
	L	95	11.8	87
	St	66	6.6	90
	Sc	65	10.2	84
	1	600	370	38
Cepacol (undiluted) two rinses daily	11	875	900	0
	12	716	476	34
	13	940	380	60
	14	628	452	28
	Re	297	31	89
Penicillin troches (5,000 units), three daily	6	1340	0.85	99.9
	7	648	0.17	99.9
	8	628	0.31	99.9
	9	1372	0.30	99.9
	10	618	0.30	99.9

medication also eliminated large numbers of lactobacilli and other oral bacteria. Attention is again called to the extremely large numbers of streptococci present in the mouths of the subjects before medication. The numbers of bacteria returned to the levels present before treatment in all subjects one or two days after medication was discontinued.

Since penicillin might be present in the saliva samples of the test subjects in sufficient concentration to inhibit the bacteria in the blood and tomato juice agar plates, suitable controls were prepared to check this possibility. For this purpose, saliva samples were collected from subjects who had large numbers of bacteria in their mouths. Control plates were then prepared, mixing suitable dilutions of these samples with samples collected from subjects

TABLE VIII

REDUCTION IN NUMBER OF LACTOBACILLI FOLLOWING DAILY USE OF TEST MEDICATIONS FOR A FIVE-DAY PERIOD

(Numbers listed in thousands. Average of five tests)

MEDICATION	SUBJECT	LACTOBACILLI BEFORE	LACTOBACILLI AFTER	PER CENT DECREASE
Glycerite of hydrogen peroxide (0.5 per cent or 1:2 concentration), two rinses daily	R	75	24	68
	4	130	110	15
	L	0	0	-
	St	403	54	86
	Sc	220	40	82
	1	2980	1460	49
Cepacol (undiluted), two rinses daily	11	640	1060	0
	12	3110	2710	13
	13	2080	1000	60
	14	3740	960	74
	Re	75	76	0
Penicillin troches (5,000 units), three daily	6	12,530	1900	85
	7	740	40	95
	8	160	70	56
	9	80	6	92
	10	100	10	90

on the penicillin test. Thus, if saliva samples from the latter group contained sufficient penicillin to inhibit the streptococci or other bacteria, the organisms in the samples of the control subjects would not develop on these plates. In no case could any such inhibition be demonstrated. When saliva from a subject not receiving medication was added to the same plate containing saliva from a patient receiving penicillin troches, the usual number of bacteria grew on the agar.

No irritation was noted following the use of the test medications, although some of the subjects using penicillin detected a coating at the base of the teeth. When the glycerite of hydrogen peroxide was used in a concentration of 0.75 per cent (1:1 dilution), certain subjects noted irritation of the mouth and gums as evidenced by chapping and loss of taste. No irritation was observed when a 1:2 dilution (0.5 per cent) was employed. A 1:3 dilution (0.37 per cent) of the glycerite of hydrogen peroxide still produced appreciable reduction of the bacterial flora.

To study further the action of penicillin on oral bacteria, five subjects dissolved two penicillin troches (5000 units) in their mouths daily for a five-day period, one in the morning after breakfast and one in the evening after dinner. Essentially the same results were obtained as are recorded in Tables VI, VII, and VIII for the subjects taking three penicillin troches each day. A very marked reduction in the numbers of streptococci and general types of mouth bacteria was obtained. Three subjects showed a definite reduction in

the number of lactobacilli and two subjects showed no reduction. One penicillin troche (5000 units) taken at 11:00 P.M. each day for a five-day period produced no diminution in the numbers of oral bacteria in the one subject tested.

During these studies it was observed that there was often an increase in the number of yeast and gram negative bacteria in the mouth following the penicillin treatment. However, no detailed study of this reaction was made. McClure and Hewitt[13] reported the inhibition of rat caries and a striking reduction in counts of oral lactobacilli by adding penicillin to rats' food and drinking water. Hill[14] found that penicillin in tooth powder reduced the lactobacillus counts and noted a subsequent increase in *Aerobacter aerogenes* in the saliva of certain subjects tested.

COMMENT

The studies reported were not undertaken to demonstrate definitely the value of certain antiseptics for the control of dental caries or other mouth diseases. It was more an attempt to determine the effect of some of the newer bactericidal agents on the oral flora. Tests should be made on many more subjects before final conclusions are made. While the penicillin troches produced a marked reduction in the oral flora, the oral lactobacilli were more resistant to its action than were the streptococci. This was in agreement with results reported by the authors[15] for in vitro tests. The possible development of penicillin-resistant strains or of other types of microorganisms in the mouth following the use of penicillin troches must also be considered. This problem is not encountered when such preparations as the glycerite of hydrogen peroxide or Cepacol are employed.

However, the results indicate that it may be possible to markedly influence or control the numbers and types of bacteria in the mouth by the use of suitable chemical agents. If this were possible, then it would point a way for the control of dental caries whether this disease is due to oral lactobacilli, streptococci, or other bacteria. This view was also recently expressed by Kesel.[16] The proceedings of Michigan Caries Conference[17] afford an excellent review of the progress being made in this important field.

SUMMARY

Tryptose glucose blood agar was found to be an excellent medium for the quantitative estimation of the oral bacteria since it affords counts on oral streptococci as well as other general types of mouth organisms. Tomato juice agar adjusted to pH 5.0 was suitable for the estimation of oral lactobacilli.

A paraffin-saliva technic similar to that described by Hadley[1] was found to be superior to a brushing and rinsing technic for the collection of representative samples for determining numbers of bacteria in the mouth. The collection of saliva over a three-minute chewing period appeared to be as satisfactory for obtaining representative samples as a shorter or longer period. However, we feel that quantitative estimation of numbers of lactobacilli or

other bacteria in the mouth should be based on total numbers of bacteria collected in the samples and not on the numbers per ml. of saliva as has been done by most investigators in the past.

The fluctuation in numbers of bacteria in the mouth at ten different periods during the day was indicated. The numbers generally decreased one-half to one hour after each meal. The largest numbers were usually found early in the morning, at noon, and late in the evening.

Under the conditions of the test, glycerite of hydrogen peroxide (0.75 per cent, 0.5 per cent, and 0.37 per cent) and Cepacol generally produced a continual reduction in the oral bacterial flora when used twice daily as a mouth rinse. A very marked reduction in the numbers of oral bacteria was obtained when two or three penicillin troches (5000 units) were dissolved in the mouth daily, the streptococci being reduced in numbers over 99 per cent.

The technical assistance of Mr. Robert P. Dudley and Mr. Ian M. Miller, formerly graduate students in the Department of Bacteriology, University of New Hampshire, with certain phases of this study is acknowledged with gratitude by the authors.

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HALITOSIS: VARIATIONS IN MOUTH AND TOTAL BREATH ODOR INTENSITY RESULTING FROM PROPHYLAXIS AND ANTISEPSIS

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THE work reported here was undertaken to secure general information concerning the intensity of odors commonly appearing on the breath, to determine the relative proportions originating in the mouth and that carried by expired air, to examine odor-intensity variation as affected by mouth hygiene and use of an antiseptic rinse,* and to make a brief study of certain habits and mouth conditions that can lead to relatively intense breath odors. In addition, data will be presented concerning the putrefaction rate of saliva in vitro and in vivo.

No data will be offered concerning physiological conditions that may be related to odor development. No subjects were included in the clinical groups who had obvious or recognizable pathological conditions. All subjects were considered as normal in regard to breath odor.

The clinical groups used were drawn from a pool comprising individuals of both sexes and ages, ranging from 18 to 60 years. The economic status included unskilled hourly rate workers, mechanics, machine operators, inspectors, foremen, all types and ranks of office personnel, executives, and laboratory personnel of various types. A special group, to be mentioned later, comprised university students of various ages.

Odor evaluations were made by application of the osmoscope, cross-checking frequently by use of the nose. Two operators cooperated constantly on odor evaluation to minimize the hazard of fatigue or the possibility of unrecognized loss of odor perception. Certain applications of the osmoscope to the evaluation of the mouth and breath odors have been reported by Fosdick.[1]

The instrument used here was that devised by Fair and Wells[2] for the evaluation of odor in potable water with a single modification, an oval rubber mouthpiece, adapting it to oral application. A glass tube through this provided for air passage.

Instrument readings given in the accompanying data have the significance shown in Table I. It has been proposed that these values be expressed in terms

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*Later repeated reference will be made to an antiseptic. In every instance this will be a saturated 27 per cent aqueous alcohol solution of thymol, menthol, eucalyptol, and methyl salicylate with benzoic acid and boric acid. The pH of this solution is 4.2. It kills a standard culture of *Staphylococcus aureus* in less than 60 seconds by the method set out in Food and Drug Administration Circular 198, U. S. Department of Agriculture.

of potential of odor (pO) following the convention in stating hydrogen-ion concentration.

The osmoscope may be used by successively increasing the dilution until the odor just vanishes, or by decreasing the dilution until the odor is first detectable. In previous applications of the osmoscope to the evaluation of mouth and breath odors,[1] an indirect method was adopted by which clean air

TABLE I

OSMOSCOPE READING (pO)	PARTS OF AIR DILUTING THE ORIGINAL SAMPLE	DILUTION RATIO
1	1	1: 2
2	3	1: 4
3	7	1: 8
4	15	1:16
5	31	1:32
6	63	1:64

was blown through the mouth, or lung air was expelled through the mouth, the air then passing through a liquid nitrogen-cooled trap, where volatile odors, water, and carbon dioxide were condensed to a solid mass. On warming to 37° C., the odor of the residual fluid was determined.

In the work under discussion, a direct method was found preferable to that devised by Fosdick[1] in that the operator sampled the mouth and expired air *directly* into the osmoscope. The dilution was decreased until the odor first became detectable. It was found that these values (direct procedure) were one point higher than those obtained by the indirect (liquid nitrogen) procedure.

Experience gained with a large group of subjects has shown that pO values of 3 or above, as determined by the direct method, are of such intensity as to be describable as "halitosis." Values below 3, even though unpleasant, are not of sufficient intensity to be so described.

It is obvious that all qualities of odor from extremely obnoxious to that of finest perfumes may yield equally high pO values. The numerical value assigned merely represents the operator's quantitative response to the intensity of the odor, and the operator must make an independent observation as to equality of the odor. Mouth and breath odors of higher intensities are never pleasant.

Rigorous attention to cleaning of the instrument was most essential as certain odors linger very persistently.

Subjects:

A pool was developed by evaluating a large group of available subjects. The very few having serious dental or undetermined systemic involvement were rejected and referred to medical attention. Sixty-five per cent of the subjects available had frequent odor levels of three or above (pO above 3). Only such subjects were used, except as reported in Sections V and VIII. Those showing pO values of less than three were rejected for later re-evaluation, as it was soon obvious that day-to-day variations existed.

The subjects comprising any group in immediate use were selected from the pool on the basis of convenience only. When necessary to divide a group into control and test sections, this was done at random. Usually it was desirable to use the same subjects for test and control.

I. Saliva Putrefaction:

Samples of saliva (stimulated by chewing paraffin) were collected from twenty subjects, none of whom had used a dentifrice or mouth rinse for several hours previously. The samples were held at 37° C. during collection. Portions of saliva so collected were diluted with 0, 2, 4, and 6 per cent, respectively, of sterile water or antiseptic and incubated at 37° centigrade. The odor values in terms of pO were averaged as shown in Table II.

TABLE II

	CONTROL				ANTISEPTIC			
	SALIVA DILUTED WITH WATER				SALIVA DILUTED WITH ANTISEPTIC			
INCUBATION	0%	2%	4%	6%	0%	2%	4%	6%
0 hr.	pO: 1.0	1.0	1.0	1.0	0.3	Odor of antiseptic		
1 hr.	2.2	2.2	2.2	2.2	1.5	0	0	0
2 hrs.	2.8	2.8	2.8	2.5	3.2	1.6	0.2	0
3 hrs.	4.5	4.5	4.5	4.2	3.9	2.1	0.5	0

II. *Saliva Putrefaction in Vivo*:

Saliva was collected from subjects who had been treated (1) with 20 c.c. of antiseptic as a 30-second mouth rinse or (2) on another day with a 20-c.c. sterile water rinse. Samples of 3 c.c. were taken just prior to the rinse, and at one-half, one-, and two-hour intervals after the rinse. The averaged data are given in Table III.

TABLE III

		WATER RINSE				ANTISEPTIC RINSE			
		INCUBATION PERIOD				INCUBATION PERIOD			
SAMPLE TAKEN	HRS:	0	1	2	3	0	1	2	3
Before rinse	pO:	2.2	2.9	3.8	4.4	2.2	3.1	4.1	4.9
½ hr. after		2.0	2.4	3.0	3.7	1.3	1.5	2.0	2.7
1 hr. after		1.9	2.4	2.9	3.2	1.5	1.5	1.8	2.3
2 hrs. after		1.9	2.4	2.8	3.8	1.6	1.5	2.1	2.7

III. *Dilution of a Mouth Rinse by Saliva*:

Antiseptic is diluted to a small degree by saliva. Obviously, the flavor, pH, alcohol content, and perhaps other characteristics of the rinse, can each influence this dilution. Twenty c.c. of antiseptic was used over a 30-second period with fifteen subjects. Alcohol content was determined and dilution calculated according to the formula:

$$\% \text{ dilution} = \frac{\% \text{ alcohol original rinse} - \% \text{ alcohol expelled rinse}}{\% \text{ alcohol original rinse.}}$$

Dilution was also estimated by determination of the volume of the recovered rinse.

IV. *Effect of Dental Prophylaxis on Odors*:

It has been suggested that decomposition of proteins in the mouth (food, mucin, etc.) could be a common cause of breath odors of high intensity. If true, removal of contaminants by dental prophylaxis could lower odor intensities. It has also been suggested that the tonsilar area could be a frequent major source of breath odors; if true, neither dental prophylaxis (IV), tongue prophylaxis (V), nor use of antiseptics as a mouth rinse (VII), should reduce breath odors substantially.

A pO value of 3 or above, in the mouth or on the total breath, was determined empirically to be so intense as to be distinctly unpleasant and describable as "halitosis."

Mouth odor values were determined by slowly drawing air through the mouth while the subject held the osmoscope by the oval rubber mouthpiece lightly but firmly with the teeth and lips. During this period the subject was careful neither to inhale nor exhale. Total breath odors were determined in the same general manner except the subject exhaled slowly through the glass tube. As so determined, mouth odors comprise only those in the mouth, while total breath odors include those originating in the mouth combined with those carried by expired air.

Subjects were selected whose pO values were all above the offensive level of 3. Each was given a thorough dental prophylaxis exactly as usually accomplished in the chair except in excluding the use of any flavored or antiseptic mouth preparations. pO values were taken immediately after prophylaxis and at one- and two-hour intervals thereafter.

Two subjects out of 17 showed no reduction in mouth level, while 6 out of 19 showed no reduction in total breath odor. The odor data were averaged for the subjects showing reduction, the corresponding data for the entire group following in parentheses in Table IV.

TABLE IV

	MOUTH ODOR	TOTAL BREATH ODOR
Before prophylaxis	pO: 3.3 (3.4)	3.6 (3.7)
Immediately after	0.5 (0.8)	1.3 (1.7)
1 hr. after	0.8 (1.1)	1.8 (2.3)
2 hrs. after	1.3 (1.6)	2.2 (2.7)

V. *Effect of Tongue Prophylaxis on Odors*:

The 17 subjects used in this series were selected from other groups where they had given indication of high odor levels due to tongue conditions. They did not represent a condition universal to the general pool from which subjects were drawn. All possessed odor levels above 3, distinctly in the unpleasant range.

Only physical means were used for prophylaxis, the tongue being scraped and swabbed without the aid of any type of flavored or antiseptic preparation. A sterile water rinse was then flushed over the tongue to remove loosened debris.

Odor determinations were made before and immediately after prophylaxis and one hour later. Eleven of the group of 17 showed reduction in odor levels, and the averaged data concerning these are shown in Table V.

TABLE V

	MOUTH ODOR	TOTAL BREATH ODOR
Before prophylaxis	pO: 3.0	3.4
Immediately after	0.5	1.7
1 hr. later	0.3	1.8

VI. *Effect of Dentifrice*:

It has been shown that the removal of debris from the mouth (IV) and tongue (V) materially reduces mouth and breath odors over a prolonged period. It was of interest to correlate the effect of general prophylaxis with dentifrice used according to a standardized procedure. Subjects were selected at random and divided into two groups: those with mouth odors above 3 and those below 3.

Two commercial pastes were used: an alkaline soap-chalk type and a synthetic detergent sulfate-phosphate type. Each subject brushed his teeth for thirty seconds, using sufficient paste to cover the length of the brush. Water was applied before, during, and after brushing, according to usual habit.

TABLE VI
DETERGENT SULFATE-PHOSPHATE PASTE

	pO UNDER 3		pO OVER 3	
	TOTAL	MOUTH	TOTAL	MOUTH
Before	1.7	1.5	3.0	3.2
After	3.6*	3.5*	4.0*	3.4*
½ hr.	1.1	1.0	1.0	1.0
1 hr.	1.6	1.7	1.5	1.8
2 hrs.	1.9	2.0	3.0	3.0

TABLE VII
SOAP-CHALK PASTE

	pO UNDER 3		pO OVER 3	
	TOTAL	MOUTH	TOTAL	MOUTH
Before	1.9	1.0	3.0	3.0
After	3.6*	3.0*	4.0*	3.9*
½ hr.	1.0	1.0	1.0	1.0
1 hr.	1.0	1.0	1.5	1.1
2 hrs.	1.6	2.0	3.5	2.4

*Flavor odor.

VII. *Effect of Antiseptic and Water Rinse on Odor*:

Five separate groups were selected at random from the pool of subjects having odor levels above 3. Each was evaluated immediately before the test started (BR), immediately after the rinse (AR), and at one, two, and three-hour interals. It had been found previously that the odor of this antiseptic rinse could not be noticed above normal mouth odors after only 20 minutes, slightly less time than dentifrice odor (VI). The several groups varied in size and averaged about 15.

The rinse, 20 c.c. of water or antiseptic, was kept in vigorous movement about the mouth for 30 seconds. These averaged data are shown in Tables VIII, IX, X, XI, and Fig. 1.

TABLE VIII
ANTISEPTIC RINSE: MOUTH ODOR

	GROUPS				
	1	2	3	4	5
BR	3.2	3.0	3.0	3.0	3.0
AR	2.5*	1.0*	1.0*	1.3*	2.5*
1 hr.	1.0	0.8	0.9	0.6	1.0
2 hrs.	1.1	1.2	1.0	0.7	0.9
3 hrs.	1.1	—	—	—	—

*Antiseptic odor.

TABLE IX
WATER RINSE: MOUTH ODOR

	GROUPS				
	1	2	3	4	5
BR	3.4	3.0	3.0	—	—
AR	2.8	2.0	2.6	—	—
1 hr.	3.0	3.0	2.9	—	—
2 hrs.	3.2	3.0	3.0	—	—

TABLE X
ANTISEPTIC: TOTAL BREATH ODOR

	GROUPS				
	1	2	3	4	5
BR	3.5	3.5	3.3	3.3	3.3
AR	4.0*	3.6*	1.5*	4.0*	4.0*
1 hr.	1.4	1.6	1.6	1.9	1.5
2 hrs.	1.8	1.8	1.5	2.1	1.6
3 hrs.	2.1	—	—	—	1.5

*Antiseptic odor.

TABLE XI
WATER RINSE: TOTAL BREATH ODOR

	GROUPS				
	1	2	3	4	5
BR	3.7	3.3	3.1	3.5	—
AR	4.0	3.0	2.8	3.5	—
1 hr.	4.0	3.0	2.9	4.0	—
2 hrs.	3.7	3.0	3.0	4.0	—

While the antiseptic rinse and mouth odor groups showed no significant number of subjects who did not secure and maintain reductions substantially under the halitosis level of 3, the total breath data indicated that as large a proportion as 22 per cent may not maintain a low level.

VIII. *Effect of Antiseptic on Morning Mouth*:

The fact that saliva putrefies rapidly during incubation suggested that stagnation of saliva in the mouth during sleeping hours can lead to measurable

odor development. To study this point, a body of male students was screened to secure a group having a particularly low daytime pO level. Fourteen subjects having an average value of 0.9 were selected.

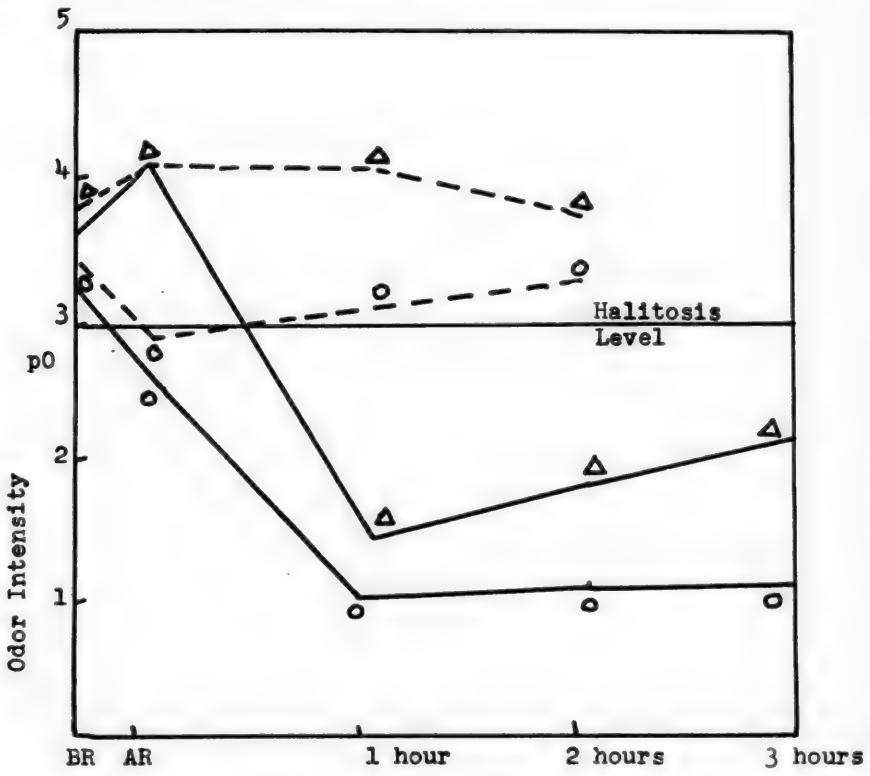

Fig. 1.—Broken line ○ — Water rinse, mouth odor.
Broken line Δ — Water rinse total breath.
Solid line ○ — Antiseptic rinse, mouth odor.
Solid line Δ — Antiseptic rinse, total breath.

The subjects were awakened individually in the morning about an hour before their normal waking hour and evaluated at once. The 10 having a pO level of 3 or more were given 20 c.c. of antiseptic as a 30-second rinse and tested again after one-half hour. During the intervening period the subjects did not eat, drink, smoke, brush the teeth, or put anything in the mouth.

On another morning a similar test was made using 20 c.c. of water as a 30-second rinse. The averaged data are shown in Table XII.

TABLE XII
MOUTH ODOR

	ANTISEPTIC RINSE	WATER RINSE
Normal daytime	0.9	0.8
Morning mouth	3.1	3.0
½ hr. after	1.1	2.3

IX. *Effect of Antiseptic on Odors due to Smoking:*

A group of cigarette, pipe, and cigar smokers were induced to stop smoking until all tobacco odor had disappeared. Curiously, smokers have a very low mouth odor other than that due to tobacco. The subjects then smoked a normal amount—two cigarettes, one pipeful, or one cigar, and the total breath pO level was determined immediately, after the usual 20 c.c. of antiseptic rinse, and later at one- and two-hour intervals. The averaged data were shown in Table XIII.

TABLE XIII

	ANTISEPTIC RINSE			WATER RINSE†		
TOTAL BREATH ODOR	CIGARETTE	PIPE	CIGAR	CIGARETTE	PIPE	CIGAR
Before rinse	4.0	4.0	5.0	4.0	4.0	5.0
After rinse	4.0*	4.0*	4.0*	3.8	3.7	5.0
1 hr.	1.8	2.0	2.0	3.1	3.7	4.0
2 hrs.	1.8	2.0	2.0	2.9	3.0	4.0
3 hrs.	—	—	—	2.3	3.0	—

*Antiseptic odor.
†All values in these three control groups are smoke in type.

X. *Resistant Types of Odors*:

In certain of the preceding tests a few subjects, apparently not abnormal in any way, resisted any single treatment—physical or chemical—given to diminish odor. About half of these subjects responded to continued daily use of the antiseptic. The other half failed to respond, however, to even prolonged treatment and could reasonably be classified as having halitosis of systemic origin, although no attempt was made to determine specific cause. The number amounted to 7 per cent of the total number of subjects having an initial odor value above 3.0.

To further examine such subjects, a group (X) was selected, who, from choice or physical inability, neglected all measures of oral hygiene. Every subject had a breath odor above the offensive level of pO 3. Twenty c.c. of antiseptic was given twice daily as a 30-second mouth rinse, treatment being continued for two weeks unless marked reduction in pO values was secured sooner. Twenty out of twenty-five such subjects secured gradually increasing odor reductions until a pO level of 2.0 or less was reached and maintained.

In contrast with the groups reported in sections I to IX, subjects in group X above were abnormal in habits of oral hygiene.

Food habits may be questioned as constituting an abnormality. It has long been recognized that certain foods can produce remarkedly persistent breath odors. The most offensive are those containing alliaceous oils. Stallard[3] has stated that garlic taken by rectum or swallowed in capsules can be detected as breath odors for as long as 60 hours. It has been proposed that breath odors resulting from chewing and swallowing garlic (1.5 gm.) arise exclusively from residues left in the mouth and that swallowing the same amounts in capsules results in the appearance of no oil (diallyl disulfide) or odor on the breath.[4]

More recently, others in agreement with Stallard, by feeding garlic through a gastric fistula, have confirmed the more usually accepted concept that garlic odors are systemic.[5] Others have reported that garlic odors have appeared on the breath of infants newly born by garlic-eating mothers.

In our study three subjects were fed 1.5 gm. garlic in capsules (washed with alcohol, then ether until odorless) and breath odors followed for 72 hours. The initial odor appeared in 30 minutes and persisted for 48 hours in two instances and 72 hours in the third. The tests were repeated, using antiseptic and various other materials as mouth rinses, without observable effect on intensity or duration of odor. Since the garlic never had contact with the mouth, the odor was systemic rather than oral in origin.

DISCUSSION

Examination of a miscellaneous group showed that sixty-five per cent frequently had mouth and breath odors above the halitosis level of 3. This level is not constant from day to day. The odor intensity of expired air (which carries lung plus mouth odor) is somewhat higher than that of the mouth alone. This should be expected since the mouth odor is only one component of the total breath odor.

SUMMARY

I. Saliva putrifies rapidly on incubation, giving rise to very objectionable odors. Dilution with water does not hinder this decomposition. Dilution with only 6 per cent of the antiseptic prevents putrefaction completely. Lesser amounts restrict decomposition.

II. The application of water as a mouth rinse does not affect rate of putrefaction of saliva collected during subsequent two hours. The use of an antiseptic rinse results in a significant reduction in putrefiability; incubation of even the two-hour sample does not give pO of 3.

III. The antiseptic mouth rinse is diluted less than 20 per cent on the average during use in the conventional manner.

IV. Complete dental prophylaxis reduces mouth and breath odors to an unobjectionable level for more than two hours. It is apparent that many breath odors are oral rather than systemic in origin.

V. Tongue prophylaxis, by mechanical means only, reduces mouth and breath odors to a low level in many instances. However, 6 out of 17 subjects did not respond.

VI. Dentifrice used in the normal manner reduces mouth and breath odors materially, the odor level returning to the objectionable level in two hours. This is not a masking effect, as the odor of the dentifrice flavor disappeared in less than one-half hour.

VII. Water rinse is without effect on mouth and breath odors. The antiseptic rinse reduces these mouth and breath odors to a low level from which no significant recovery takes place within three hours. This is not a masking

effect, as the odor of the rinse disappears in twenty minutes. The effect of the antiseptic is prolonged significantly beyond that of prophylaxis or dentifrice.

VIII. Stagnation of saliva in the mouth overnight can result in an odor intensity of objectionable level, even though the subject has a normally low daytime level. Water rinse does not reduce this odor materially. The antiseptic rinse removes the odor.

IX. Tobacco odors are materially reduced by the antiseptic rinse but not by a water rinse. Smokers appear to have a low mouth or breath odor other than that of tobacco. Cigars produce highest odor levels. Cigarette odors are most readily reduced to a low level.

X. There are certain resistant types of odors, such as that due to garlic, that are systemic in origin and are not affected by water or the antiseptic rinse.

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PHILADELPHIA SECTION
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INFLAMMATION. *Valy Menkin, M.D., Temple University.* Inflammation is a manifestation of cellular injury producing leucotaxine (a leucocytosis-prompting factor), necrosin, pyrexin (a leucopenic factor), leucoperin, and glucose in the course of the reaction. The presence of these substances explain many of the phenomena associated with the reaction.

Leucotaxine, which has been isolated in crystalline form, is responsible for capillary permeability. Its effects are local only.

The leucocytosis-promoting factor is a polypeptide attached to a globulin. Its presence results in hyperplasia of bone marrow and the release into the blood stream of many juvenile leucocytes.

Necrosin, a euglobulin, causes lymphatic blockade and injury to the vessel walls. Its effects are not limited to the site of experimental injection.

Pyrexin, a glucopeptide, is responsible for a rise in temperature. It is linked with leucoperin which traps leucocytes in the lungs, liver, and spleen.

Inflammation is accompanied by fibrosis, disturbance of fluid exchange, localization of the irritant, and migration of leucocytes.

*Held at the School of Dentistry, University of Pennsylvania, Dr. J. T. Gore, presiding.